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THROMBOSIS

AND

ANTICOAGULANT THERAPY

Proceedings of a Symposium arranged by Professor P. A. Owren, Professor R. B. Hunter, and Dr W. Walker, and held in QUEEN'S COLLEGE, DUNDEE, on 29 and 30 September, and 1 October, 1960.



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UNIVERSITY OF ST ANDREWS

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FOREWORD

The conference on thrombosis and anticoagulant therapy sponsored by the University of St Andrews was, to my mind, an excellent example of the value of concentration in discussion. The subject was timely. Though representative of a number of European countries and of the United States, the total number of delegates was small enough to allow them to be housed under one roof. The proceedings were conducted in one language. The university and the civic authorities were so hospitable that it was clear that neither had overlooked the original meaning of the word symposium.

Very properly, the conference began by attempting to define the phenomena to be discussed in terms of pathology, and it emerged that information on these matters was by no means complete. However, a new method of producing artificial thrombi and a new technique for identifying plasminogen activator in the tissues held promise of providing more information about the factors which preserve the fluidity of blood. That therapeutic efforts to ensure fluidity of blood were desirable in a variety of clinical conditions was generally agreed and, on the whole, Thrombotest was accepted as the most generally useful method of controlling the administration of anticoagulant drugs. The ideal, a test to disclose both tendency to thrombosis and tendency to bleeding, seemed as elusive as ever.

Although the precise way in which anticoagulant drugs produce their good results when given for long periods remained unsettled, the choice of the types of patient to receive life-long anticoagulant therapy was not a matter of controversy, nor were priorities within these types. That such therapy might take a substantial contribution to any national economy was generally agreed and the administrative machinery for securing this proved a fascinating topic.

The relatively recent use of extracorporeal circulations provided a new field for the use of anticoagulants, which was duly explored. And, finally, it was obvious that the possibility of producing new synthetic anticoagulants was by no means exhausted.

One of the advantages of a symposium is that it is not burdened by any obligation to reach final conclusions, or to make recommendations. What a symposium can do is to accomplish — strictly for the time being — certain objectives; to confirm good methods of treatment and good techniques in research; to agree to abandon those which have been unfruitful; to choose those which are promising enough to be retained after improvement; and, above all, to suggest new avenues of approach Perusal of these proceedings will make it clear that, in the pursuit of these objectives, the symposium succeeded uncommonly well.

James Learmonth.

CONTENTS

Current concepts of blood coagulation in relation to vascular disease.

The organisation and laboratory control of anticoagulant therapy.

The use of anticoagulants in myocardial infarction, in peripheral and cerebral vascular disease, and in prophylaxis.

CONTENTS

Thrombosis, Blood Coagulation And Atheroma—

A CRITICAL APPRAISAL

J. R. A. MITCHELL—M.R.C. Clinical Research Fellow, Department of Hæmatology, Radcliffe Infirmary, Oxford

Anticoagulants are being used with ever- [illegible]

[illegible] offer an explanation based on two assumptions. Firstly, that thrombosis is intra-vascular clotting, and secondly, that the in vivo action of anticoagulants is the same as their in vitro action. Unfortunately, the two premises on which this train of thought depends are not valid.

Whatever thrombosis is, it is not simple intravascular clotting, and naked-eye examination of the end products of the two processes convinces us of this. An intravascular clot is dark red, shiny, soft [illegible]

[illegible] cell itself.

[illegible]

[illegible] fibrin coagulum, was a relatively late event, and [illegible]

[illegible]

[illegible] enough calcium to deal with the transfused blood and yet it does not clot). Laboratory tests of blood coagulation may not, therefore, give any indication of the state of affairs in the living vessesls

[illegible]

[illegible] and studied the staining properties of the fibrin formed. They found that unless they added 1 g.% [illegible]

[illegible] observations), and until this has been resolved, the confident identification of fibrin by the fluorescent antibody technique must be accepted with caution.

[illegible]

[illegible] chemical environment will determine the staining reaction rather than the mere presence of the blood component in question. There may be a place for the fluorescent antibody technique in platelet identification, and we are studying this problem.

Finally, I shall discuss briefly the relationship [illegible]

[illegible] predisposes to coronary occlusion by thrombus, resulting in myocardial infarction (Fig. 4). However, each of these assumptions can be assailed.

The relationship between infarction and [illegible]

[illegible] survey of atheroma, occlusion and ischæmic heart disease and find that in our series, in recent infarctions

Figure 1

Figure 2

Figure 1—Section through clot, showing even distribution of red cells and fine fibrin strands. Mallory P.T.A H x 200.

Figure 2—Section through experimental rabbit pulmonary thrombus showing fibrin laminæ, white cells, amorphous platelet debris, and paucity of red cells Mallory P.T.A H x 200.

Figure 3—Section through mural thrombus showing platelet aggregates surrounded by white cells. H. & E. x 200



Figure 3

MYOCARDIAL INFARCTION = CORONARY THROMBOSIS = CORONARY ATHEROMA = GENERAL ATHEROMA

"CLASSICAL"

DUGUID

RABBIT "ATHEROMA"

HIGH BLOOD CHOLESTEROL

RABBIT CHOLESTEROL POISONING

Figure 4 — Diagram showing supposed inter-relationship between cardiac infarction and vessel disease, and (dotted lines) the false analogy between human lipid findings and the results of rabbit experiments

Figure 4

with definite cardiac necrosis, occlusion is found in over 80%, whereas in patients who have died suddenly the inc[illegible]

[illegible] infarcts.

The second equation was questioned by Morris (1951) who showed that, despite the rising tide of cardiac infarction, the amount of coronary atheroma had not increased over the years in post-mortem examinations at the London Hospital, whereas the frequency of coronary occlusion had. Robertson (1959) found that in racial groups with widely differing infarct rates such as Jamaicans and the inhabitants of New Orleans the coronary atheroma pattern may be very similar, whereas the number [illegible]

[illegible] the years in one population group, or in one racial group as compared with another, then it should leave behind in the patients who recover an increased amount of atheroma).

If thrombosis is the critical factor in the increasing frequency of cardiac infarction, then perhaps [illegible] we sh[illegible] botic [illegible] wall a[illegible] is sug[illegible] Regis[illegible] mona[illegible] of in[illegible] virtua[illegible] incren[illegible] this tr[illegible]

T[illegible] —how[illegible] levels [illegible]

[illegible] in low and high infarct rate populations (Jones [illegible]

The three equations shown in Figure 4 are still incomplete and even if the inter-relationships do exist, is the direction of the "reaction" a forward one from atheroma to thrombosis or is it Duguid's "back reaction" from thrombosis to atheroma?

Figure 5

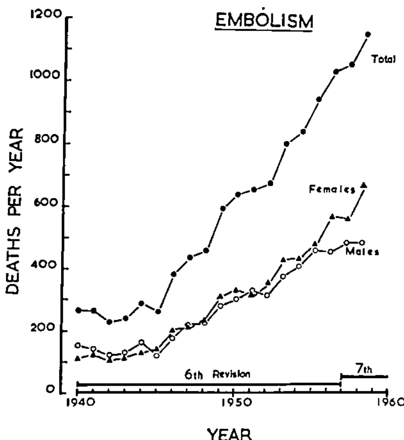


Figure 5—Annual death rate from pulmonary embolism and pulmonary infarction (Registrar-General's Statistical Review of England and Wales 1940-1958)

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Thrombosis And Haemorrhagic Diathesis

M. MATTER — Krankenanstalt Neumünster, Zurich

Thrombosis and hæmorrhagic diathesis are considered to be opposite processes. Thrombosis is certainly rare in thrombocytopænia and severe coagulopathies. This is one reason why anticoagulants are used in thrombosis. Another is the belief that thrombosis is the consequence of hypercoagulability.

As thrombosis and its prevention are the main subjects of this conference, I shall concentrate on thrombogenesis, and show that study of the coagulation mechanism and of coagulation defects has greatly contributed to the understanding of this process. I shall not discuss the possibly important role the fibrinolytic system plays in regulating physiological and pathological thrombus formation. Stress and the administration of ACTH and cortisone lead to an imbalance of this mechanism which may be of great importance in the pathogenesis of venous and arterial thrombosis.

In severe hæmophilia, a marked deficiency of a coagulation factor leads to a prolonged clotting time and delayed and incomplete thrombus formation. It is easy to measure such defects with simple methods In thrombosis one would by contrast

formation is a more dynamic process and the coagulation factors may rapidly change. Only frequent measurements of blood samples may show the sequence of events

But the "whole blood clotting time" also,

meas
Fig.
speec
hatched area.

Hypercoagulative states show a shortening of r and k. With this sensitive method it has been possible to measure increased coagulability in many

bosis the values of r and k return to normal or even show decreased coagulability (15). Even this method does not detect a tendency to or an existing thrombosis with certainty. As changes in the concentration of coagulation factors and variations of r and k in

decreased coagulability. If great amounts of tissue juice enter the circulation as in premature separation of the placenta, fibrinogen, prothrombin, factor V and platelets may be so reduced by intravascular coagulation that a severe hæmorrhagic diathesis follows. Fig 2 shows the TEG of such a patient (A); no thrombus formation occurs; B is the normal control, C a mixture of the patient's and normal blood. If small amounts of thomboplastin enter the circulation massive thrombosis does not develop, but the following changes are seen in a sequence of

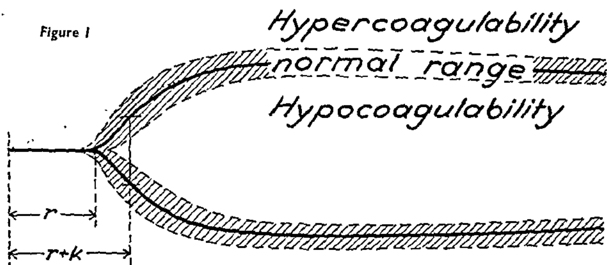
state (C). The same changes are noted before, during and two days after normal delivery. They may be the result of the entrance of small amounts of tissue juice from the placenta into the circulation (6).

The same changes are often found during

thrombosis (3). As arterial thrombosis is usually

At the site of an endothelial arterial lesion, the first visible change supposedly consists of the agglutination of platelets (Fig. 4). This may take only a few seconds and is independent of the

Figure 1



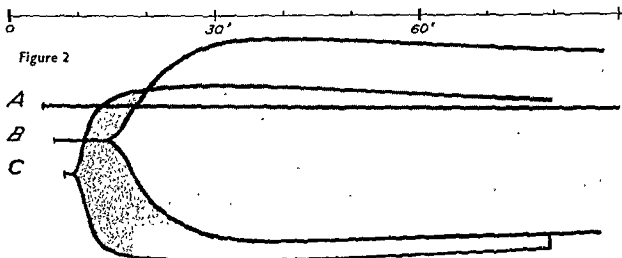
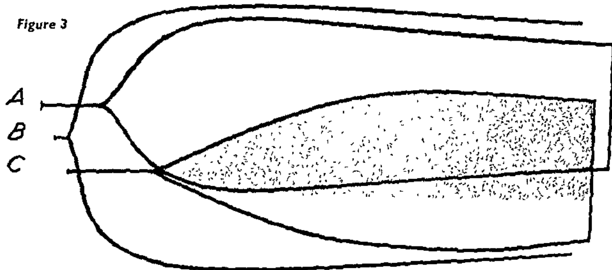


Figure 2

Figure 3



Extrinsic System

Tissue Thromboplastin

Ca''

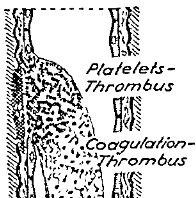
F V

F VII

F X

Active Thromboplastin

Prothrombin → Thrombin → Fibrin ← Fibrinogen



Intrinsic System

Platelet Factor III

Ca''

F V

F VIII

F IX

F X

PTA

(Hageman-F.)

Active Thromboplastin

Fibrin

Fibrinogen

Thrombin ← Prothrombin

Figure 4

Figure 5

PLASMA | COAGULATION | SERUM

F VIII, X, (PTA)

100 %

50 %

0 %

ACTIVITY

F IX, Hageman-F.

F I, II, V, VIII

TIME →

(14). Platelet factor 3 reacts with Ca, the antihæmophilic factors, PTA, Hageman factor and factor X.

During coagulation fibrinogen, prothrombin, factor V and factor VIII are consumed. The activity of Ca, factor VII, factor X and PTA remains

contains thus a thrombotic accelerator, which is present also in the sera of patients with the following coagulopathies: factor V, factor VII, factor VIII and factor X deficiency (19).

The serum of factor IX and Hageman factor deficiency does not contain this accelerator. The activity is decreased in PTA deficiency and in patients treated with dicoumarol for at least one week. Factors IX and Hageman, inactive in plasma, become activated when the blood is exposed to wettable surfaces such as glass (12, 13). Their activation at the site of the endothelial lesion may be one of the first changes initiating coagulation in thrombosis.

The experiments of Wessler favour the hypothesis that the production of serum is very important in thrombogenesis. It agrees with the clinical observation that a major thrombotic process, particularly venous, usually follows minor thrombosis due to trauma including surgery and delivery.

thromboplastin in the intima

Wessler found heparin efficient in preventing experimental thrombosis whereas dicoumarol and its derivatives showed decreased activity in the serum only after a week of treatment (20). This effect probably is due to factor IX deficiency, which only develops after this period of therapy.

We are not yet able to produce isolated Hageman factor deficiency for therapeutic purposes It would never lead to hæmorrhage, and, therefore, would be the ideal antithrombotic treatment.

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Factors Influencing The Formation Of Artificial Thrombi

J. C. F. POOLE—Sir William Dunn School of Pathology, University of Oxford

blood when poured into a glass tube or some such container, while thrombosis is taken to mean the deposition on the inside of a blood vessel wall of solid material from the blood. Some people use the words indiscriminately. But the distinction is important because the microscopical appearances of a thrombus and a clot are different.

A thrombus has an elaborate structure made up of two different kinds of material. One

masses appear as structureless areas. That this consists of platelets is not immediately obvious but in fact there has never been much doubt about it. Several workers in the eighteen-eighties observed the formation of thrombi in living vessels; they could see platelets being deposited and when they cut sections through such regions areas of amorphous material were seen. The matter was settled by Levene & Levene (1957), who studied the structure of human thrombi with the electron microscope, and confirmed that in these areas, where no structure is shown in ordinary sections, platelets very closely packed together can be made out.

This basic structure is common to all naturally occurring thrombi, but there are many variations. The proportion of the platelet and white cell material to the red cell and fibrin material varies greatly. The platelet material may comprise nearly the whole structure or only a small part. In a common type it is concentrated in a small area called the white head attached to the vessel wall while the greater part consists of a red tail of red cells and fibrin, extending downstream from the white head. The platelet and leucocyte masses may be loosely packed branching coralline structures or form streaks or layers.

The structure of a blood clot is quite different. The formed elements of the blood are distributed

It is obvious that a new technical approach is needed to provide a bridge between studies of blood clotting in the test tube and of thrombosis in the body: in short, a method for producing

If Chandler's procedure is modified slightly by sticking the ends of the tube together to make a smooth joint it is possible to form structures which resemble natural thrombi even more closely (Poole, 1959). In such an apparatus the blood does not clot at all in the ordinary sense. Instead, a small solid body forms just behind the advancing edge of the column of blood and floats round in the blood for an indefinite period. A section through one of these bodies shows a structure similar to that of certain kinds of thrombus with a white head and a red tail.

head. Then, after an interval, the red tail forms suddenly and the structure is fully formed.

The effect of free fatty acids was investigated because of the known effect of fatty acids on the clotting of plasma (Poole, 1955). Long chain saturated fatty acids in low concentration shorten the clotting time of plasma, while short chain saturated fatty acids and long chain unsaturated fatty acids do not. When these observations were made nobody knew whether free fatty acids occurred in human blood. Robinson, Harris, Poole & Jeffries (1955) showed that the usual range of free fatty acid in the plasma of healthy young men was about 0.3 to about 2 milliquivalents per litre. Stearic acid added to plasma in that concentration gives a maximal effect on the clotting time and some effect with smaller

concentrations in which the active fatty acids produce a shortening of the thrombus formation time are again small and within the physiological fluctuations. Of the straight-chain saturated fatty acids,

palmitic acid, C 16, there is a marked shortening and with longer chain acids from stearic acid, C 18, onwards there is an even more marked effect. The long chain fatty acids tested included one of the unnatural series, the odd-number acids, C 23, and this behaved much like its neighbours in the series.

The four long chain unsaturated fatty acids tested are inactive or nearly so. They are oleic acid, elaidic acid, ricinoleic acid, and arachidonic acid.

Of the active acids the most abundant in nature is stearic acid and the investigation has started of the effect of simple modifications of the molecule. Substitution of one methyl group reduces and of two abolishes activity. A hydroxyl group makes little if any difference.

When artificial thrombi are formed in the presence of an active fatty acid they are not only formed more rapidly but are also larger. Whether in life the size of a thrombus or the speed of its formation is the more important, in this experimental situation the answer is the same

In vivo, a fatty meal leads to a rise of free fatty acid concentration in the blood, but there is much controversy about whether or not fatty meals produce hypercoagulability. Conflicting claims have been made and it is difficult at present to hold any clear views on the matter.

The effect of adrenaline injection and stress is less obscure. Cannon & Gray (1914) observed shortened whole blood clotting time in cats after injections of adrenaline. Connor confirmed this

treated rats of the same strain in exactly the same way and found an increase in the free fatty acid content of their blood, so here again in vivo hypercoagulability and increased free fatty acids go together.

Further speculation is unprofitable as the data are as yet incomplete. But when a system which produces objects very like natural thrombi is influenced in this way by free fatty acids in concentrations which occur in the circulating blood, then one must take seriously the possibility that some such mechanism might operate in vivo.

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The Recalcified Plasma Clotting Time As A Measure Of Blood Coagulability

H. W. FULLERTON and G A. McDONALD—

Department of Medicine, University of Aberdeen

One approach to the problem of the patho- ...is has been to ... vitro by a wide ... he large amount ... these lines, the position is still far from ... fact, the results

A further difficulty is that the process of coagu-

...formation

In recent years we have carried out several studies using the recalcified plasma clotting (R.P.C.) time as a measure of coagulability, and our first

objective was to standardise the technique and to find out whether or not it gave reproducible results. Details of the technique have been given in a previous communication (McDonald and Fullerton, 1958 a) and all the steps have always been undertaken in exactly the same way by one of us (G.A.M.). We consider this to be of great importance if accurate results are to be achieved and we would stress that none of the work on which this communication is based has been delegated to technicians.

[illegible]

greatest difference between the two arms was only [illegible] is 3 5 seconds. [illegible] shortens the [illegible] I which gives [illegible] arms in 14 patients, the venepuncture being satisfactory in one arm, while in the other it was unsatisfactory in that tissue trauma was produced inadvertently or deliberately. The readings in the samples obtained by unsatisfactory venepuncture were consistently much shorter, the mean difference being 28 3 seconds. Accordingly we have always discarded blood samples obtained by unsatisfactory venepuncture.

We have previously shown that a high-fat breakfast containing approximately 85 g. animal-fat (2½ oz. bacon, 2 eggs, 1-1½ oz. butter, tea with milk and sugar) results in a marked shortening of the R.P.C. time a few hours after the meal (McDonald and Fullerton, 1958, a and b). We have found a similar effect after approximately the same amount of vegetable-fat mainly as margarine (McDonald and Fullerton, 1958 a), but our observations in this communication are confined to the results obtained with the standard animal-fat meal and they are conveniently discussed under two headings

1—*The effect of physical activity on the acceleration of the R P C. time after ingestion of the high-fat meal.*

Observations have been made in two groups of in-patients, none of whom suffered from cardiac disability, or received any drug likely to affect coagulability.

Group A.

The findings in the first group of 11 patients [illegible]

Group B.

[illegible]

Observations in two groups of ambulant subjects have shown distinct differences from the previous two groups of in-patients.

Group C.

This comprised 10 out-patients who had had [illegible]

higher (mean 126 7 seconds) than in either group of in-patients, and second, that the reduction 3½ hours after the high-fat meal is less (mean 16.7 seconds).

Group D.

This consisted of 23 students who reached hospital by walking short distances and by using public or private transport. After the high-fat breakfast they attended lectures and clinical instruction, walking a few hundred yards in the intervals between them. The findings are very similar to those in Group C (Table 3). The mean fasting R.P.C. time is 139.7 seconds and the mean reduction 3½ hours after ingestion of the meal is 18.4 seconds.

Group E.

Comprised 16 of the 23 students of Group D. In or[illegible] start [illegible] only [illegible] after [illegible] remaining 16 reported to hospital as before, and [illegible]

meal was only 7.2 seconds whereas it was 23.8 [illegible]

time which occurred after the fatty meal when the subjects were mainly sedentary.

In subjects at rest in bed (Groups A and B) the fasting R.P.C. time is short and is much accelerated after a high-fat intake. Both of these factors could be regarded as favo[illegible]

[illegible] 1953).

2—*The effect of phenindione on the increase in blood coagulability following a high-fat intake.*

This has been studied in two groups of patients as follows:

Group F.

10 in-patients with recent cardiac infarction at rest in bed and treated with phenindione for periods varying between 4 and 18 days at the time the experiments were conducted.

Group G.

10 out-patients with previous cardiac infarction who had had therapeutic doses of phenindione for periods varying between 12 and 45 months.

In both groups the one-stage "prothrombin time" had been maintained at 2 to 2½ times the control level and the last dose of phenindione was taken in the evening before the test day. In all the subjects in both groups blood samples were removed before and 3½ hours after the breakfast containing 85 g. animal fat.

It is seen that in Group F (Table 4) the mean fasting R.P.C. time is slightly prolonged (147.1 seconds) as a result of the administration of phenindione, but the interesting point is that the mean acceleration 3½ hours after the high-fat meal is only 3 6 seconds. This is in marked contrast to the findings in Groups A and B (non-cardiac in-patients receiving no phenindione) where the mean reduction in the R.P.C time was more than 30 seconds

In Group G (Table 4) the fasting R.P.C. *time* is again slightly prolonged (mean 147 3 seconds) and at 3½ hours after the high-fat meal the expected acceleration is not seen, the mean of the readings being only 2 3 seconds less than the mean fasting value. In respect of physical activity this group is comparable to Group C (Table 3), i.e. the control group of out-patients with previous cardiac infarction receiving 1 mg. phenindione daily, in which the mean R.P.C. time 3½ hours after the high-fat meal is 16 7 seconds shorter than the mean fasting value.

Therapeutic doses of phenindione virtually abolish the acceleration of the R P C. time which occurs after the ingestion of a high-fat meal in subjects not receiving this drug. So far as we are aware this action of phenindione has not been [illegible]

The practical importance of this action of [illegible]

[illegible]

TABLE 1

SIMULTANEOUS R.P.C. TIMES (SECS) IN BOTH ARMS IN 14 SUBJECTS: VENEPUNCTURE UNSATISFACTORY IN ONE ARM.

	Satisfactory Arm	*Unsatisfactory* Arm	Difference
Means	120 7	92.4	28.3

TABLE 2

MEAN R P.C. TIMES (SECS.) BEFORE AND AFTER HIGH-FAT MEAL.

GROUP A: 11 IN-PATIENTS (NON-CARDIAC).

Fasting	3 hour	Difference	4 hour	Difference
114	75	38.7	79	35 5

TABLE 3

MEAN R.P.C. TIMES (SECS.) BEFORE AND AFTER HIGH-FAT MEAL.

	Fasting	3½ hour	Difference
Group B: 10 In-patients (non-cardiac).	111.6	80 4	31.2
Group C: 10 Out-patients (old cardiac infarction 1 mg P.).	126.7	110	16.7
Group D: 23 Students, "sedentary" day.	139 7	121.3	18 4
Group E: 16 Students, "active" day	133.1	125.9	7.2

TABLE 4

MEAN R P C. TIMES (SECS) BEFORE AND AFTER HIGH-FAT MEAL

THERAPEUTIC DOSES OF PHENINDIONE.

	Fasting	3½ Hour.	Difference
Group F: 10 In-patients, recent cardiac infarction.	147.1	143.5	3 6
Group G: 10 Out-patients, old cardiac infarction.	147.3	145	2.3

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Studies On Fibrinolysis

SOME INTER-RELATIONSHIPS BETWEEN CLOTTING AND FIBRINOLYSIS

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The theoretical background of thrombosis and its treatment is related to the phenomena of coagulation and fibrinolysis. Coagulation research is the "basic science" in which progress may be of great importance for adequate prophylaxis and treatment of thrombotic disease.

Coagulation and fibrinolysis are intimately connected. Their biological role is antagonistic. An increase in coagulability favours thrombus formation, the activation of the fibrinolytic system counteracts it.

The intimate mechanism of these mutual interactions is not completely known. We do not know, we only guess which particular components may act simultaneously in both systems.

The following are the significant findings concerning coagulation factors which influence the fibrinolytic system:

(1) Fibrinogen and fibrin possess considerable

theory of clot dissolution in vivo postulated by Sherry et al. (1959)

(2) The fibrinolytic activity of thrombin in vitro was found by Guest and Ware (1950). Kowarzyk (1952) described a "thrombin protease" connected with thrombin. Seegers and Landaburu (1957) demonstrated that one of the different molecules of thrombin (thrombin E) possesses fibrinolytic activity.

Interrelationship between thrombin and the fibrinolytic system in vivo was demonstrated by Kudriaszov and Ulytina (1958). After an injection of thrombin into animals the fibrinolytic activity increases so rapidly that the blood does not clot.

(3) Kowarzyk and Rechnic (1957) found that the euglobulin fibrinolysis is activated by removal of plasmin inhibitor during thrombin formation from prothrombin ("thrombogenetic disinhibition of fibrinolysis").

(4) Calcium ions added in appropriate concentration activate the coagulation system and also fibrinolysis in euglobulin (Kowalski et al., 1959).

euglobulin fibrinolysis time is shown in Fig. 1. It

EUGLOBULIN FIBRINOLYSIS AND „CONTACT FACTOR"

INCUBATION MIXTURE (30 MIN, t 37 °C)	LYSIS TIME (HOURS)
SERUM EUGLOBULIN + SALINE	20,0
EUGLOBULIN OBTAINED FROM SERUM SHAKEN WITH KAOLIN (KAOLIN IN EUGLOBULIN) + SALINE	3,25
EUGLOBULIN OBTAINED FROM SERUM SHAKEN WITH KAOLIN (KAOLIN REMOVED) + SALINE	10,0
ALCALINE ELUATE FROM KAOLIN + SALINE	11,0
ALCALINE ELUATE FROM KAOLIN + SERUM EUGLOBULIN	2,5

Figure 1

activit

F

evaluation of these phenomena.

After this short review of coagulation factors influencing the fibrinolytic system some data concerning the influence of plasmin (fibrinolysin) on clotting factors may be summarised.

Plasmin inhibits all coagulation stages by destroying some coagulation factors. Its action on fibrinogen and fibrin is very well known

FIBRINOLYTIC ACTIVITY OF EUGLOBULIN PREPARED OF SERA SHAKEN WITH KAOLIN AND CELITE

Figure 2

SPECIES	LYSIS TIME (MIN)					
	SPONTANEOUS ACTIVITY			SK – ACTIVATED		
	CONTROL SERUM	KAOLIN TREATED SERUM	CELITE TREATED SERUM	CONTROL SERUM	KAOLIN TREATED SERUM	CELITE TREATED SERUM
HUMAN	300	23	34	4	1.5	4
BOVINE	> 1000	300	>1000	>1000	20	195
SHEEP	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
HORSE	1000	55	180	210	20	25
RABBIT	960	140	960	10	5	10
GUINEA – PIG	7	1	2	—	—	—

ACTIVITY OF BLOOD CLOTTING FACTORS AFTER INCUBATION WITH PLASMIN (+ 37 °C)

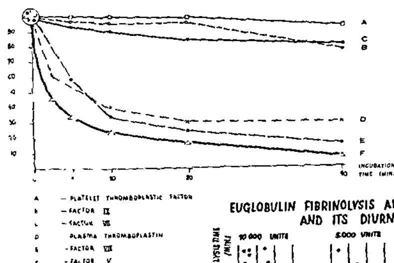


Figure 3

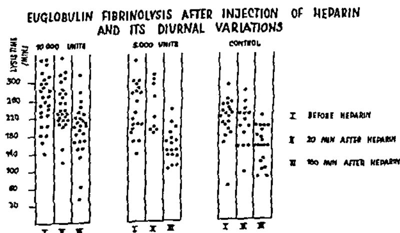


Figure 4

Soulier et al. (1956), using whole plasma, reported that in vitro plasmin destroys Factor V, VII, prothrombin, AHG, Christmas Factor and complement, in order of decreasing sensitivity. Niewiarowski and Latallo (1957), who used purified factors, found that plasmin inactivated AHG, Factor V, and plasma thromboplastin, but does not influence the Christmas Factor, Factor VII and the thromboplastic activity of platelets (Fig. 3).

There is also experimental evidence that the fibrin stabilising factor (FSF) is destroyed by plasmin (Buluk 1959).

At least two different inhibitors of coagulation appear during the proteolysis of fibrinogen: anti-thrombin VI (Niewiarowski and Kowalski 1956; Niewiarowski et al. 1959) and inhibitor of thromboplastin formation, I.T.F. (Niewiarowski et al. 1959). They inhibit thromboplastin formation, thrombin action on fibrinogen and fibrin monomer polymerisation (Kowalski 1959).

The pharmacological treatment of thrombosis can be performed with three kinds of substance: (1) heparin or heparinoids; (2) dicoumarol and its derivatives; (3) plasmin and other fibrinolytic substances. Each of these substances may interfere with the naturally occurring clotting or fibrinolytic processes.

(1) Heparin. The actions of heparin on blood clotting are described in a separate paper. Concerning the influence of heparin on the fibrinolytic system, the literature is contradictory (von Kaulla and MacDonald, 1958). We injected heparin in a single dose of 5000 or 10,000 units into normal human subjects. Blood samples were drawn before injection of heparin and 20 minutes or 3 hours after the injection. The fibrinolytic activity was estimated using the euglobulin method (Fig. 4). No significant changes were observed 20 minutes after injection of heparin. An activation of fibrinolysis was noted 180 minutes after injection of 5000 or 10,000 units. However, this activation is of the same order of magnitude as the diurnal variations of fibrinolysis (Fearnley et al. *1957*; *Kaniak et al. 1959*). Similarly, it was shown in these experiments that heparin has no influence, at 20 and 180 minutes, on the plasminogen and antiplasmin levels.

From these investigations it seems that heparin has no significant influence on the fibrinolytic system in vivo.

(2) Dicoumarol derivatives. Their influence on [illegible]

plasminogen level is not influenced by these anticoagulants. No activation of fibrinolysis in whole plasma was observed (Halse 1948; Wirecki 1958). Our group investigated the influence of the dicoumarol derivatives Tromexan, Sintrom, and Marcoumar on plasma fibrinolytic activity (Niewiarowska and Wegrzynowicz 1959). They were studied in a number of patients with coronary

PLASMA FIBRINOLYTIC SYSTEM IN PATIENTS DURING LONGTERM ANTICOAGULANT THERAPY

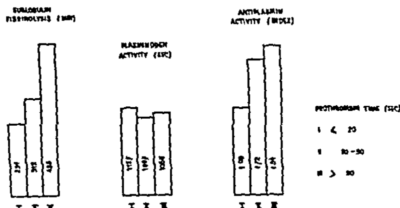


Figure 5

THE COAGULATION AND FIBRINOLYTIC SYSTEM IN RABBITS AFTER A PELENTAN ADMINISTRATION

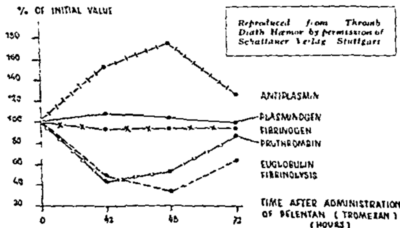


Figure 6

INFLUENCE OF PLASMIN (10 ml/kg. OF WEIGHT)ON THE BLOOD COAGULATION SYSTEM IN THE DOG

	DOG 1		DOG 2		DOG 3	
	I	II	I	II	I	II
Fibrinogen mg/ml	1,68	1,68	1,56	1,0	1,68	1 44
Fibrinolysis in Total Plasma	10% ×	10% ×	0% ×	100% ××	0% ×	100% ×
Clotting Time (Lee-White)	3'40"	6'55"	1'30"	4'20"	3'10"	7'30"
Recalcination Time	50"	60"	46"	246"	52"	—
Thrombin Time	31"	>6'	17"	>6'	17"	>6'
Antithrombin VI	18"	32"	21"	>6'	—	—
Prothrombin Time	9"	9"	9"	16,5"	8"	16"
Factor V	13"	14"	12"	12"	12"	12"
Factor VII	18"	18"	19"	18"	20"	21"
Factor VIII (Ahg)	100%	100%	100%	100%	100%	100%
Factor IX (Christmas)	100%	110%	100%	115%	100%	95%

I BEFORE INFUSION
II 50 MINUTES AFTER INFUSION
× AFTER 24 HOURS
×× AFTER 20 MINUTES

Figure 7

thrombosis on long-term anticoagulant therapy and in rabbits treated with a single dose

These anticoagulants have no significant effect on fibrinogen and plasminogen levels. They all cause a prolongation of the euglobulin fibrinolysis time, and an increase of antiplasmin level. These two changes may be correlated with the prolongation of the prothrombin time (Figs. 5 and 6).

It has been suggested that a substance inhibiting fibrinolysis activation increases in the plasma of patients treated with dicoumarol derivatives. This substance could be removed by kaolin or celite (*Niewiarowska and Wegrzynowicz 1960).*

(3) The purpose of treatment with proteolytic enzymes is the dissolution of clots. We injected SK-activated plasmin into dogs and cats and examined the coagulation system. Contrary to results in vitro no changes in clotting factors other than fibrinogen were observed after plasmin injection The fibrinogen level decreases extremely fast in cats, while in dogs this phenomenon is slower (Fig. 7).

The finding of an indefinitely prolonged thrombin time and greatly increased antithrombin

proteolytic enzymes is related to the level of antithrombin VI in the circulation.

We consider the thrombin time to be the most effective test for the control of treatment with proteolytic enzymes. Deutsch and Fischer (1960) are of the same opinion.

After an excessive dose of proteolytic enzymes

merisation and that protamine sulphate and other agents may overcome this effect (Kopec et al. 1960). These findings are not discussed here in detail They provide a rational background for treatment with proteolytic enzymes, its appropriate control and prophylaxis of complications

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The Tissue Activator Of Plasminogen And Thrombosis

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Since thrombosis is a local phenomenon it may be caused by failure of a local fibrinolytic mechanism. Therefore, it seemed necessary to investigate the distribution of fibrinolytic activity within the tissues.

The chief fibrinolytic substance in tissues is an activator of plasminogen (*Astrup and Permin*, 1947). This substance can be detected and assayed by the fibrin plate method (Permin 1947, Astrup and Albrechtsen, 1957). The method is to incubate fragments of tissue or drops of tissue extract on a

method was modified for microscopic examination (Todd, 1958, 1959).

In the new method, a histological section of fresh tissue is placed on a very thin layer of fibrin, and incubated. During incubation structures containing plasminogen activator cause lysis of the adjacent fibrin. After incubation, the preparation

In all tissues the areas of fibrinolysis appears to be related to blood vessels and in most cases the veins show more activity than the arteries (Fig 2).

In the liver and spleen the amount of fibrinolytic activity is small and it is almost entirely related to large veins both portal and systemic (Fig. 3). The *pulmonary arteries are very active* (Figs. 4 and 5) and so are the main pulmonary veins

Large blood vessels show most activity in relation to their vasa vasorum (Fig 6), but zones of fibrinolysis can often be seen in relation to the intima, and to fragments of endothelium lying free in the lumen (Figs. 7 and 8.)

The latter observation suggested that it was the endothelium that contained the plasminogen activator. This was tested by scraping cells off the intima of a large vein and incubating them in a fibrin film. Under these conditions, endothelium caused enough fibrinolysis to account for all the activity seen in sections (Fig. 9).

The fibrinolytic activity produced by arteries is usually very much less than that seen in veins.

this reaction involves 3 stages:—1—The conversion of a plasminogen proactivator to an activator by *a kinase (streptokinase)*. *2—Conversion of* plasminogen into plasmin by activator. 3—Lysis of fibrin by plasmin.

from the circulating plasma but this is so loosely attached that even two washings will remove it. These results suggest that the fibrinolytic mechanism in the endothelium is in the form of a latent system which is set in motion by change in local conditions, or perhaps by exposure to some humoral agent which acts as a "kinase".

Three observations are of interest in a search for the local conditions or substances which may produce activation of this fibrinolytic mechanism. The first concerns the distribution of the fibrinolytic activity throughout the tissues of the vascular

arteries its effect is negligible. This type of mechan-

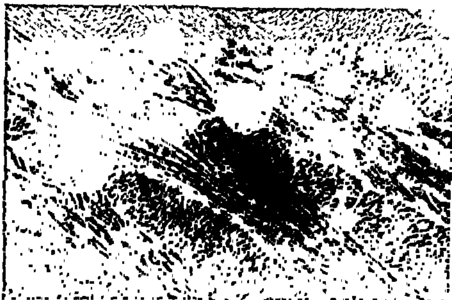


Figure 1—Fibrinolysis autograph of muscle. The pale areas are gaps in the fibrin background indicating fibrinolysis due to the presence of plasminogen activator in the tissues

Hæmalum × 76

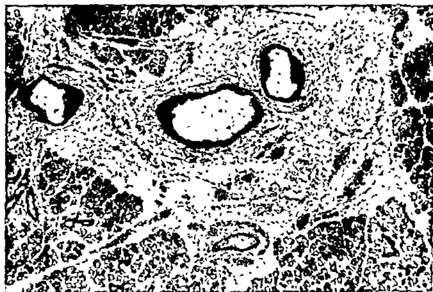


Figure 2—Salivary Gland showing fibrinolytic activity round blood vessels The ducts are inactive

Hæmalum × 30



Figure 3—Liver. The activity is confined to large veins and their vasa vasorum

Hæmalum × 30

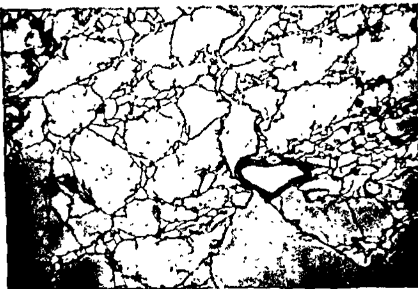


Figure 4—**Lung.** The fibrinolytic activity is related to pulmonary arteries.

Hæmalum × 30

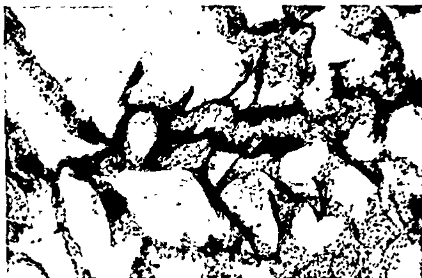


Figure 5 — **Lung.** Showing lysis round a pulmonary artery.

Hæmalum × 76



Figure 6—**Aorta.** The fibrinolytic activity is related to the vasa vasorum. The intima (left) is inactive

Hæmalum × 30

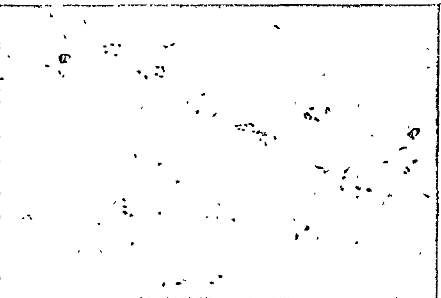


Figure 7—Vena Cava showing activity in relation to the vasa vasorum, to intima, and to cellular fragments in the lumen   Hæmalum × 45

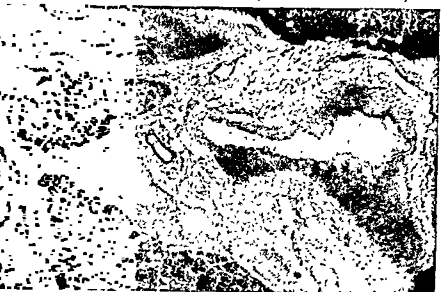
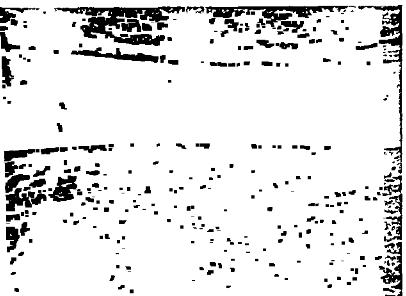


Figure 9—Endothelial cells from a vein. They show marked fibrinolytic activity   Hæmalum × 96

Hæmalum × 76   *Figure 8*—Pulmonary Artery showing fibrinolytic activity round desquamating fragments of endothelium



Hæmalum × 30   *Figure 10*—Salivary Gland showing moderate activity in the large central artery and normal venous activity.





Figure 11—The wall of a vein from a leg amputated under bloodless conditions Note the platelet clumps in the areas of lysis

Hæmalum × 76

ism would explain the gradient of endothelial fibrinolytic activity from the venous to the arterial parts of the circulation. Incidentally, a similar gradient of fibrinolytic activity has already been described in the blood itself, that from the veins being more active than that from the arteries (Fearnley and Ferguson, 1957).

The second set of observations related to the behaviour of blood and tissue in limbs which have been ischæmic. Kwaan and McFadzean (1956)

samples are taken the distribution of fibrinolytic activity is such as to suggest that the most active blood is that which has stagnated in the capillaries and small venules I was able to study tissues from a limb amputated for sarcoma under "bloodless" surgical conditions using an Esmarch bandage

paralleled by an increase in the amount of plasminogen activator in the vascular endothelium

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ndothelium

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the latent activator mechanism It was noted in the preparations from the ischæmic limb that the active endothelial cells were often accompanied by clumps of platelets, suggesting that they, too, had been damaged.

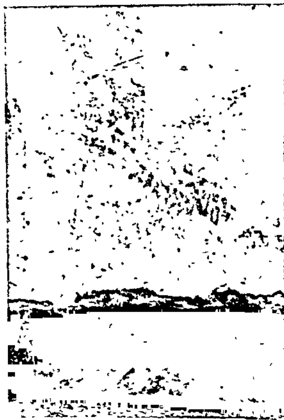


Figure 12—Artery from an amputated leg. There is unusual activity in the arterial contents (above) which include detached endothelium.

Hæmalum × 76

These observations suggest two ways in which the fibrinolytic mechanism in endothelium may be set in motion Firstly, by damage, and secondly,

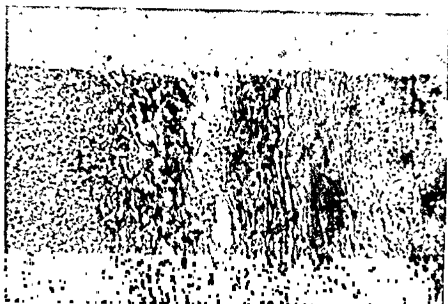


Figure 13—Thrombosed vein showing fibrinolytic activity *in the vasa vasorum and in* channels in the thrombus

Hæmalum × 30

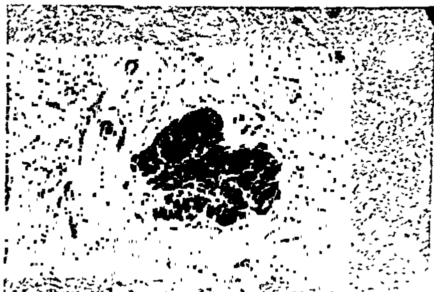


Figure 14—Metastatic prostatic carcinoma in the seminal vesicle. The fibrinolytic *activity is related to the* surrounding tissue rather than to the clump of cancer cells.

Hæmalum × 30

by some humoral substance which may come from the tissues into the venous blood. It has previously been shown by Astrup and Buluk (1960) that the ... It appears

Brinkhous and ... (Opie and Barker, 1907) in the blood normally keep the rate of these reactions at a low level (Astrup, 1956b). Alteration in these humoral factors may determine the outcome of local damage. A balance ... mechanisms enables ... and an ... gulation ... rease in

fibrinolysis can produce a dangerous hæmorrhagic state, presumably because fibrin acts as a part of the blood-tissue barrier, as Nolf (1908b) suggested.

These two extremes can be illustrated by histological preparation. A section of a thrombosed vein shows that where *the thrombus is adherent* to the vessel wall no fibrinolytic activity can be detected in the intima. Activity can, however, be ... detached from the wall

(Fig. 14) show that it is the tissue round the tumour rather than the carcinoma itself which produces the excessive activity.

To Summarise. 1—The fibrinolytic activity of tissues is concentrated in the endothelium of blood vessels. 2—The endothelium of veins is much more active than that of arteries. 3—Arterial endothelium may be activated by exposure to streptokinase. 4—There is some evidence to suggest that the fibrinolytic activity of endothelium may be increased by damage and perhaps by some substance which accumulates in the veins from the tissues. 5—It is suggested that local fibrinolytic mechanisms such as the one described are important in the pathogenesis of abnormal thrombosis.

APPENDIX (Materials and Methods).

1—*Tissues.* Biopsy and post-mortem human tissues varying in age from a few minutes to 48 hours may be used. If the material is to stored it is rapidly frozen and kept in the ice chest.

Fibrinogen. Bovine Plasma Fraction I ("Armour") which is supplied as a freeze dried powder is dissolved in modified Michælis buffer

contain 20 units per ml.

Cellophane. Ordinary commercial cellophane is used. It must be "wettable" and have neither

2—*Preparation of Fibrin Film.*

This is made as a thin layer of fibrin on a cellophane backing using the following technique.

A sheet of plate glass is accurately levelled by

poured into the dish and spread quickly with a glass rod and left to coagulate. 1 ml of fibrinogen

in a moist chamber at 4°C. For use they are placed, cellophane side down, on a microscope slide.

3—*Tissue Sections* are cut on the freezing slide microtome using the Schultz Braun cold knife technique (Romeis, 1948). The sections are taken straight on to the fibrin film.

4—*Hautchen Preparations* of endothelium are made as follows: A square of tissue is cut from the wall of a large blood vessel and washed in saline. After being allowed to drain briefly it is placed, endothelium downwards, on a microscope slide and the excess fluid at the edges is dried off by touching with the edge of a filterpaper. The slide is then placed on the chuck of the freezing microtome and the area bearing the vessel is chilled with two

5—*Incubation.* 15-30 minutes incubation at 37°C is usually sufficient to demonstrate fibrinolytic activity. The preparations are enclosed in a "moist chamber" during this stage.

6—*Fixation* The preparations are exposed to the vapour of boiling formaldehyde solution for 30 seconds and are then placed in formol saline solution for 1 hour. During this stage the fibrin becomes detached from the cellophane. Fibrin and section are then treated together in the same way as a "floated out" section.

7—*Staining.* The preparations are stained for 5 minutes in full strength Harris's hæmatoxylin and differentiated overnight in distilled water. They are "blued" by exposure to ammonia vapour.

8—*Mounting.* They are mounted in glycerine jelly on glass slides.

9—*Fibrinolytic Activity of Whole Blood.*

By the method of Fearnley, Balmforth and Fearnley (1956). The activity was expressed as the

$$\frac{1}{\text{lysis time in minutes}} \times 10{,}000$$

ACKNOWLEDGMENTS

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DISCUSSION

it is certainly not arterial spasm, for there is no stimulus for this. Besides, arterial blocks in various sites are local, probably from a platelet build-up, and the cause is in the vessel wall. Also, did Dr Todd's method with frozen sections exclude the activation of lysis in the processing?

Professor OWREN—Mitchell's investigations again emphasise the lack of correlation between arteriosclerosis and clinical disease. The rising mortality seems not to be caused by increasing atherosclerosis. An increased tendency to thrombosis must be considered. The decline in post-operative thrombosis and coronary disease in Norway during the war is consistent with this. The effect of diet must be studied further, and I agree that it is not simply a problem in coagulation: factors independent of it are recognised. We have studied mainly hæmostasis after injury to small vessels, but certain reactions are the same in arterial thrombosis though the trigger mechanisms may differ. In both the wound surface and the vessel wall are decisive. Wessler's and Poole's experiments, however, are different, because clot-promoting factors in the blood play the major role. They may give important information for venous thrombosis and the problem of hypercoagulability, but it must be applied cautiously to arterial thrombosis.

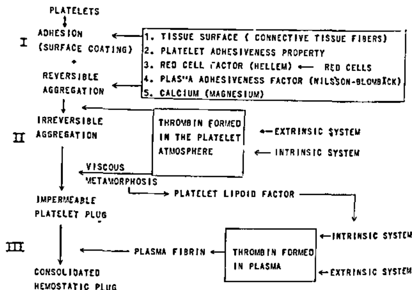


Figure 1—The hæmostatic mechanism

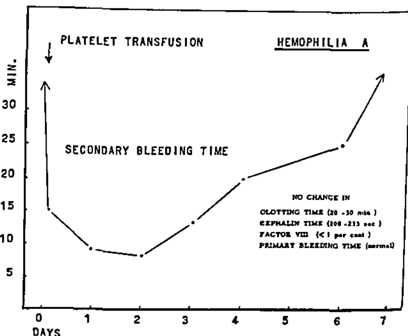


Figure 2—The effect on the secondary bleeding time in hæmophilia of transfusion of normal platelets (Platelets from 2 l of plasma were given)

In the hæmostatic mechanism (Fig 1) factors 1-4 are essential since the primary bleeding time and platelet adhesiveness in vivo (Borchgrevinck, Acta Med Scand., 1960) are altered in hæmorrhagic telangiectasia, thrombosthenia, severe anæmia and Von Willebrand's disease The tissue substrate for platelet adhesion is the collagen and elastic fibres (Hugues). Platelets adhere loosely or not at all to cholesterol and fat—relevant to the trigger mechanism of thrombosis secondary to atheroma.

The red cell factor (factor R of Hellem, Scand. J. Clin. Lab. Invest., Suppl 12, 1960) may be an important trigger. Platelets in red cell-free plasma are not adhesive. Addition of washed or trypsin-treated red cells increases adhesiveness to glass parallel to the hæmatocrit value A protein-free concentrate of factor R has been prepared, and causes

a platelet thrombus. Bleeding in the vessel wall may also be significant.

The second stage is platelet fusion by viscous metamorphosis, presumably caused by thrombin. [illegible]

Moreover, plasma concentrations must be reduced to about 15%.

Sudden death from electrical instability (ven- [illegible] with advancing coronary disease.

[illegible]

not believe that atheroma is thrombotic in origin, [illegible]

injected irritant substances into the arterial lumen while the blood flow was temporarily arrested, but this resulted in thrombosis only rarely and unpredictably. To produce thrombosis regularly, it was necessary to reduce the rate of flow through the segment; thrombosis was then almost invariable. The shock of a myocardial infarct may produce the circumstances leading to thrombus formation I, therefore, welcome Dr Mitchell's finding that occlusive thrombus is found more often in patients living for some days after the ischæmic episode, compared with patients dying suddenly.

Dr SCHWARTZ — The low incidence of occlusive thrombus in Professor Montgomery's series is probably due to the age of the infarcts. It is much less common in older infarcts.

Professor HILL — The patients who die instantly at the onset of myocardial necrosis are important [illegible]

Relative acute ischæmia may explain infarction without thrombosis. Similarly, the liability to sudden death of patients with grossly hypertrophied ventricles is explicable by anoxia favouring ventricular fibrillation · the capillary blood supply does not keep pace with the enlarging muscle fibres.

Dr SHARP — When platelet-rich plasma clots, the platelets clump some time before fibrin forms For 1-2 minutes after clumping it can be reversed [illegible]

In Poole's illustrations, the platelets in the thrombus formed discreet clumps of uniform size. [illegible] mass.

Platelets on clumping increase in volume roughly fourfold. This also is of interest in thrombus formation.

Dr SCHWARTZ — In the "sudden cardiac death" group there is no coronary thrombosis and the degree of atheroma is unimportant (current Oxford studies). This group should be excluded from discussions of coronary occlusion in myocardial [illegible] covered.

Quite frequently at autopsy widespread thrombotic occlusions are found in many arteries. This suggests a systemic thrombotic tendency.

There is much autopsy and clinical support for Mitchell's views. Coronary and femoral artery thrombosis together have been found to occur during adequate anticoagulant treatment. A man aged 46 developed mesenteric angina due to aortic thrombosis he had primary polycythæmia with a platelet count over 3 million, and his aorta was free from atheroma

Dr MITCHELL — What reason is there for attributing "sudden death" to heart disease at all? As in Beck's experiments, these deaths might possibly be due to embolic occlusion of a twig vessel from a proximal atheromatous plague, giving ventriculour fibrillation Cardiac infarction cannot be due to atheroma alone: the deterioration is too rapid. Thrombus or embolus is the most likely explanation.

Dr VERSTRAETE — Did Professor Fullerton find a strict parallelism between the Quick time and the R.P.C. time, and was the prolongation of the latter found even when the Quick time was not in the therapeutic range?

Professor FULLERTON — Our experiments were all done on patients in the therapeutic range or showing no effect at all.

Dr HARTERT — Did Dr Poole find increased viscosity shortly before visible clotting started? With a similar device, the ring viscometer, we could not distinguish between increased viscosity and the beginning of real clot formation.

Dr POOLE — When fibrin forms, the level of the advancing edge becomes still lower, quite quickly, and thus is how we measure thrombus formation time. The change in the position of the column of blood is probably due to friction between the fibrin and the walls of the tube.

Dr VERSTRAETE —

... of the trace of thrombin needed for thromboplastin generation: other tests fail to reveal its activity.

Dr NIEWIAROWSKI —
menta
inhibit
substa
(throm
test)
... and
VI
bo-
nti-
... VI can be obtained free of I.T.G., and electrophoretically pure I.T.G. has been prepared by Lipinski in our group. I.T.G. may, in fact, act by inhibition of ...

... Verstraete's experiments, moreover, components of the clotting system other than thrombin may have been eliminated.

With regard to Dr Todd's findings, Kowalski et al. (Blood, 1958, 8, 436), using entirely different methods, found that the activity of plasminogen or plasminogen proactivator is very high in organs rich in connective tissue.

Dr MATTER — The increased antiplasmin activity in ...

Professor DONALD — With regard to Dr Todd's experiment in which he activated separated arterial ...

... endothelial cells?

Dr TODD — Red cells and leucocytes lose their activity, derived from the plasma, after two washings, whereas the substance is firmly attached to arterial endothelium. Serosal cells behave like arterial cells. Bladder epithelium is inactive.

In answer to Professor Pickering, the activity of the tissues decreases with age and also with fixation. Only fixation in cold 70% ethanol is of any use. The activity found in tissues is inversely proportional to the amount of autolysis.

It might also be suggested that bacterial activity could be responsible for the phenomenon. This is unlikely, because (1) sterile biopsy material shows it; (2) no bacteria are seen in digestion zones; (3) there is no lag phase before lysis begins; (4) addition of antibiotics and antiseptics to the system does not abolish the reaction.

The Organisation Of Large Scale Life-Long Anticoagulant Therapy

P. A. OWREN—Rikshospitalet, Oslo

Introduction.

Anticoagulant therapy has been violently debated [illegible]

[illegible]

two problems:

(1) Which patient should be treated?

(2) How should the control be organised?

The therapy was first given to patients with acute diseases, and was stopped after a few weeks However [illegible]

So far, most physicians and laboratories have experience in managing only a few hundreds of patients However, because of the incidence especially of atherosclerotic disease in the western countries, we must now face the practical problem of making permanent anticoagulant therapy available to the great number of patients who need it. This means that we must develop an organisation for large-scale therapy.

Indications for Life-Long Anticoagulant Therapy.

The general structure of [illegible]

[illegible] limited to atherosclerotic disease and rheumatic heart disease because patients with other diseases giving indication for life-long anticoagulant therapy are comparatively few.

We classify the patients in two main groups. In the first group anticoagulants have marked effect on mortality and disablement: this is our first priority group. In the second group the effect of anticoagulant therapy is less well established.

The high priority group includes five sub-groups.

(a) Chronic rheumatic heart disease. Figs. 1 and 2 show the effectiveness of prophylaxis in [illegible]

[illegible]

(b) [illegible]

[illegible] often important—work. Our first study of angina pectoris comprised 471 patients admitted to the University Clinic of Oslo. All patients were given anticoagulants, and the mortality was only 3 6% per year in the treatment period, totalling 1400 years (Owren 1954). The usual mortality in patients not so treated is 8-10% per year In a second study of 275 patients Waaler (1957) found that patients with a short history had a mortality of less than 1% during the first year (fig 3). In the group with long duration of symptoms before therapy, averaging 3.5 years in this material, the mortality was about 6% per year, which is not significantly different from the usual mortality of 8-10% (fig. 4) Based on these results we organised a third study which has been conducted by Dr Borchgrevink (1960). In order to [illegible]

[illegible] therapeutic range, as expressed by the P & P method and consequently also by the new Thrombotest method, must be below 50%

(c) Myocardial infarction In this large group we select patients with only one attack, with no angina, or with angina for less than 2 years Fig. 6 shows the survival curve for non-selected patients on anticoagulant therapy, our own group of 308 patients and Bjerkelund's group of 119 patients. Survival curves for 3 groups of non-treated patients

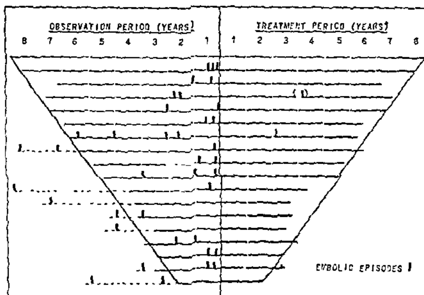
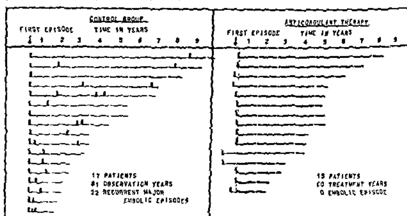
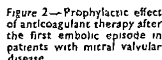


Figure 1 — The effectiveness of anticoagulant treatment in patients with mitral valvular disease and recurrent embolism prior to therapy




Figure 2 — Prophylactic effect of anticoagulant therapy after the first embolic episode in patients with mitral valvular disease

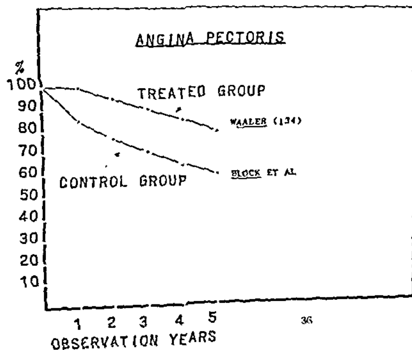


Figure 3 — Effect of anticoagulant therapy in angina pectoris with short duration of symptoms prior to therapy.

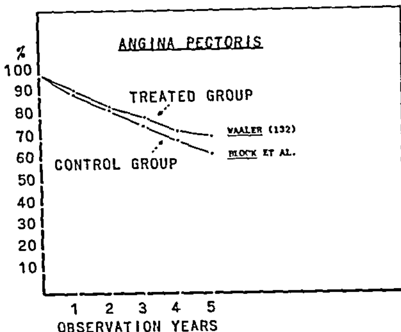


Figure 4—Effect of anticoagulant therapy in angina pectoris with long duration of symptoms (average 3 5 years) prior to therapy

Figure 5—Effect of anticoagulant therapy in patients below 70 years of age with angina pectoris of less than 2 years duration and without myocardial infarction.

	P & P LEVEL	
	50 %	20 %
NO. OF PATIENTS	68	70
MYOCARDIAL INFARCTIONS	9	1
CARDIAC DEATHS	6	1
MORTALITY IN % PER YEAR	8	1.3

are included for comparison. The number of patients with more than one infarction before therapy was relatively small in these series, and definite conclusions regarding the prognosis in such cases could not be drawn. The figures, however, suggest that new infarctions were more frequent in patients with more than one infarction prior to therapy, both in the treated groups and in the control groups. Bjerkelund states that "anticoagulant therapy does not seem to have protected these patients much" (Bjerkelund 1957, p. 134). It is also logical to assume that the effectiveness of anticoagulant therapy is highest at an early stage of the disease. There is no reason to believe that the antithrombotic effect per se is less in advanced disease. The difference in results is presumably caused by a higher frequency of non-thrombotic deaths in advanced disease, that is, death from congestive heart failure and sudden death from acute ventricular fibrillation

(d) Atherosclerosis in the legs without signs or symptoms of coronary or cerebral atherosclerotic disease. All these patients have high priority, not because of the local disease, but because they need protection against coronary death. A high proportion of these patients die of coronary disease or of cerebral arterial disease. At our outpatient clinic, 140 patients with thrombosing atherosclerosis in the lower extremities have been on anticoagulant therapy for an average of 4 years (Osvik, 1960). The total mortality has been 3.75% per year, with 2.7% of cardiovascular disease. A study at Ulleval Hospital in Oslo of a large group of similar patients not treated with anticoagulants showed a total mortality of 8% per year. We must not forget that atherosclerotic disease is a generalised disease, and should be treated as such.

(e) Intermittent insufficiency of the cerebral circulation, presumably due to thrombosis, is the last category on our high priority list A decisive study [illegible] is stil [illegible] us th[illegible] The r[illegible], however.

Our list of second priority cases includes three main categories: rheumatic heart disease without

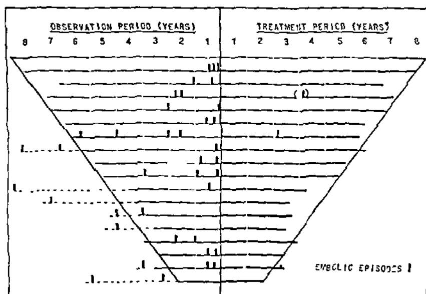


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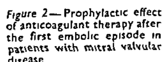
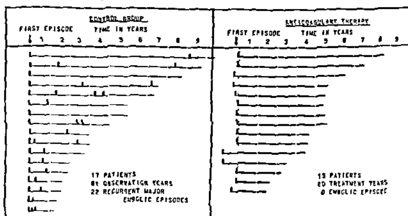


Figure 2 — Prophylactic effect of anticoagulant therapy after the first embolic episode in patients with mitral valvular disease



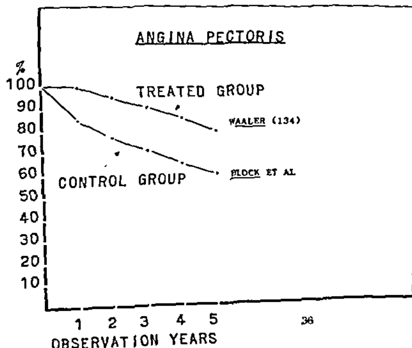


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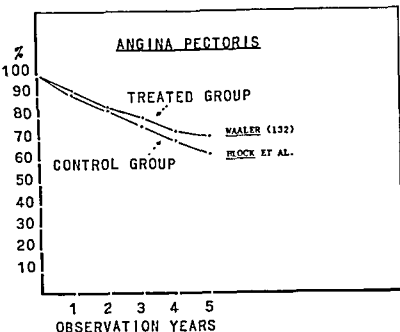


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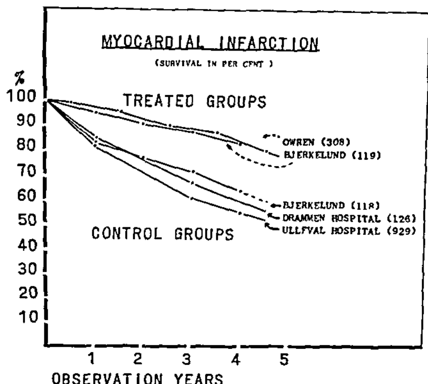


Figure 6—The effect of life-long anticoagulant therapy on mortality of survivors of acute myocardial infarction

embolism, angina pectoris of more than 2 years duration and two or more myocardial infarctions. For these groups there is only suggestive evidence that anticoagulant therapy decreases mortality. Therefore, it is not justified to insist on treatment for all these patients. Each patient should be considered an individual problem However, further research using the technique of well-controlled clinical trials must explore the statistical value of anticoagulants in such patients

Size of the Problem.

Our question is now. how big a practical problem is it to organise anticoagulant therapy for all patients in the high priority group and for selected patients in the low priority group?

The death rate in Norway from chronic rheumatic heart disease is about ten per hundred thousand If we assume an average life expectancy of 15 to 20 years after the appearance of clinical symptoms, the morbidity would be 150-200 per 100,000 Probably not more than 25% of these are candidates for protection by anticoagulants, that is, about 50 per 100,000.

The death rate for atherosclerotic and degenerative heart disease in Norway is 173 per 100,000 This includes also most cases with arteriosclerosis [illegible]

[illegible]

probably includes about 1/5 of these or about 150 per 100,000 in Norway. If all new patients receive therapy, the number will increase to 800 per 100,000, or somewhat more if one allows for increased life expectancy due to treatment.

Thus, we may conclude that roughly 200 per 100,000 at present, rising to 1000 per 100,000 or 1% of the population in the next 10 years need to be on anticoagulant therapy. There are many assumptions and approximations in this calculation, but it appears realistic to us.

The morbidity of atherosclerotic disease is somewhat higher in Great Britain than in Norway, but still the size of the problem is not overwhelming

Thus, then, will be the problem. to maintain as many as 0.2% up to 1% of the population on safe and effective anticoagulant therapy. How should this be done?

Basic Problems of Anticoagulant Therapy.

I must first analyse the basic problems of life-long therapy. To make my points clear, I shall arbitrarily divide these problems in two groups. [illegible] and has 2 items. other forms of therapy and psychological problems.

[illegible] that some doctors tend to give anticoagulants to patients who do not need the treatment, such as

patients with cardiac neurosis, with uncomplicated [illegible]

When we started long-term anticoagulant therapy at the University Clinic in Oslo 12 years ago, we used the P & P method for control. After 10 years, with more than 100,000 tests, we decided to modify it. The P & P test has one theoretical [illegible]

come these difficulties, in order to make anticoagulant therapy simple and yet safe.

The P & P test was first modified to make it sensitive to all four factors reduced by the oral [illegible]

with thousands of patients, the capillary test saves much time and is more convenient for the patient. [illegible]

The new test is simple, accurate and takes only about 2 minutes. It has been in general use in Norway for 1½ years. It can be done by any office nurse or [illegible]

(3) Administration of anticoagulants is apparently an extremely simple matter. Nevertheless, experience shows that the result is often bad; the patients frequently get too small and fluctuating doses. Both patient and doctor may be responsible for this.

The patient must be thoroughly instructed. It is necessary to sit down with him and explain in detail what the purpose is, what the dangers are, and that you expect him to follow directions absolutely. Never must he forget to take his pills, and never must he change the prescribed dosage.

The doctor must know the rules of dosage. They are few and simple, but often overlooked. Personal experience is the best teacher, and most doctors need two months before they master this art. To obtain good results, the doctor must be accurate in details and have a high degree of responsibility.

[illegible]

Organisation.

[illegible]

explanations or repeat the test at once. This is an important detail. In this way charts are not mixed up, and it constantly reminds the patient that great care is necessary. If the patient has no problems, he goes back to work at once. The same afternoon all charts are reviewed by the doctor who makes necessary adjustments in the dosage. This is again an important detail. Such adjustments must never [illegible]

doctor about, he can see the doctor when the test is finished.

In this organisation the technical and medical problems are handled by the same doctor, who [illegible]

less than half an hour each day reviewing the charts. The patients spend a few minutes in the laboratory every month. Finally, this organisation is inexpensive: the cost of reagent is at present about 9 shillings and of technical assistance about 8 shillings a year per patient.

When the laboratory is part of a hospital, it is sometimes necessary to separate the technical and medical problems. The laboratory then takes care [illegible]

capacity for anticoagulant therapy in our country [illegible]

enced doctor.

Many patients cannot conveniently come to such a laboratory for control. Their problem can be solved in two ways. Either they can have their practitioner send the blood to the laboratory, or the practitioner can do the test in his own office. It is perfectly feasible for him to do this. With a little experience and cheap equipment he can obtain reliable results. This solution would also combine the technical and medical responsibility, which is an advantage. Experience shows, however, that the result of this organisation is often not good This is not because there are any difficult problems, but simply because most doctors need experience before they do the job well. This is especially true for the dosage, which needs more experience than the performance of the test.

We therefore recommend the following solution. If the general practitioner has a number of such patients, and if he is willing to devote himself

do the whole work alone.

In this discussion I have tried to outline the general principles, rather than to make specific proposals. It is not possible to build up one system for all patients, since the system must be modified to meet local needs. It is a waste of time to discuss the theory of centralisation versus decentralisation, because this is an entirely practical problem.

In conclusion, I will stress that this is a large, but not overwhelming problem. It can be solved,

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The Laboratory Detection Of Thrombosing And Of Bleeding Tendencies

HELLMUT HARTERT—Med Univ Hospital, Heidelberg, West Germany

The problem with which I would like to deal arose in a very practical way in our laboratory. We were used to controlling anticoagulant therapy by thromboelastography (TEG). We believe this to be a safe method of control, since phases of pronounced tendency to thrombosis during therapy

method.

For practical reasons we changed our method of drawing blood one year ago Before this we took

With this change to oxalated full blood we noticed a very pronounced *sensitivity for increased coagulability*. This was indicated by a greater shortening of the clotting time in thromboses, in cases with lung embolism and also in myocardial reinfarction

With citrated plasma we had found that

being value diffi-culty of statistical comparison, a great difference in favour of the oxalated full blood method.

This difference in sensitivity seemed not to be caused by the addition of the red cells but by the

We were very pleased about the increased sensitivity of this test for a thrombosing tendency until we noticed that now the sensitivity for a bleeding tendency had gone This was indicated by several heavy bleeding accidents, where the Quick test constantly showed values below 5 %. The TEG on the contrary failed with the oxalated blood method to indicate a bleeding tendency.

It has always been our opinion that the good sensitivity of TEG in the control of anticoagulant therapy and especially in detecting a thrombosing tendency was due to its special sensitivity for the thromboplastic factors of the plasma. The importance of estimating thromboplastic activity in anticoagulant therapy has also been indicated by the attempts of several authors to include one or more thrombo-

plastic factors in routine determination of the prothrombin complex.

[illegible]

Let us assume that in the TEG study of the [illegible]

[illegible] tendency can apparently not be detected, if the influence of the extrinsic clotting system, as it is determined in the Quick test, becomes more prevalent by changing the method. This means the impossibility of a joint determination of thrombosing and of bleeding tendencies in one test, if both are to attain the highest possible sensitivity.

[illegible] hand there is no explanation why the therapeutic reduction of clotting factors must be so relatively high to escape thrombosis.

In contrast to this, the bleeding tendency seems to be strictly correlated with a clotting defect—except where there is a toxic defect of the capillaries. [illegible]

[illegible] gives a good cross-section of the plasma proteins, but does not reveal for example important antibodies, which can cause death.

Anticoagulant therapy is a rather crude method. It does the same as bulwarks on the slopes against rolling avalanches. Would it not be better to catch the sparrow before he starts the avalanche—or prevent the snow from becoming so dangerous? But [illegible]

It is not my intention to undermine any existing or proposed method. I should like to ask rather how a control system could be devised which would both avoid bleeding and prevent thrombo-embolism.

Comparative Study Of Control Methods For Anticoagulant Therapy

W. ZOLLINGER — Krankenhaus, Neumunster, Zurich
With the technical assistance of Miss L Muller

The methods used were: Quick's test, whole blood clotting time, recalcification time, thrombelastography and the heparin tolerance test. Many authors believe that it is safe to use Quick's method alone. Others believe in simultaneous use of the methods mentioned.

The reliability and reproducibility of any particular method depend on several important factors:

1—The techniques have to be simple, so that in any laboratory the same results can be obtained by different workers.
2—The interpretation of the results should be by an experienced physician taking into consideration the anticoagulant used, whether long or short acting.
3—The greatest problem is the choice of thromboplastin.

The ideal thromboplastin should fulfil the following criteria:

(a) Simplicity in use.
(b) *Sensitivity to all* factors depressed by the anticoagulant.
(c) Insensitivity to factors not influenced.
(d) Reproducible results by different workers at different laboratories.
(e) Same results with plasma, decalcified whole venous or capillary blood.
(f) Should not give too long a clotting time.
(g) Moderate cost.

It should be available anywhere anticoagulant therapy is practised. Such a preparation can be of animal or human origin.

We studied the commercial preparations

firms.

Different thromboplastins were first compared using plasma. There was a good relation between *Quick's method and the Thrombotest, especially* within the therapeutic range. The mean value by Quick's method is about 10% higher than by Thrombotest, because different diluents are used for the calibration curves, for Thrombotest Seitz-filtered plasma and for Quick's method Michaelis buffer

The same picture was obtained when Thrombo*kinase Geigy was compared with* Thrombotest. When human brain thromboplastin was compared with Thrombokinase Geigy a good correlation was obtained in the therapeutic range. Thrombokinase Geigy is rather insensitive to variations in factor VII (4) Since factor VII is rapidly depressed at

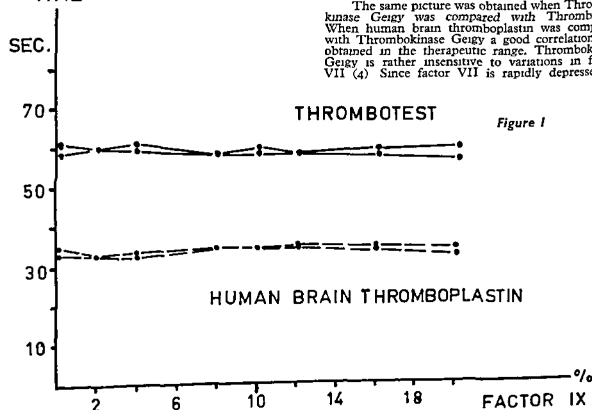


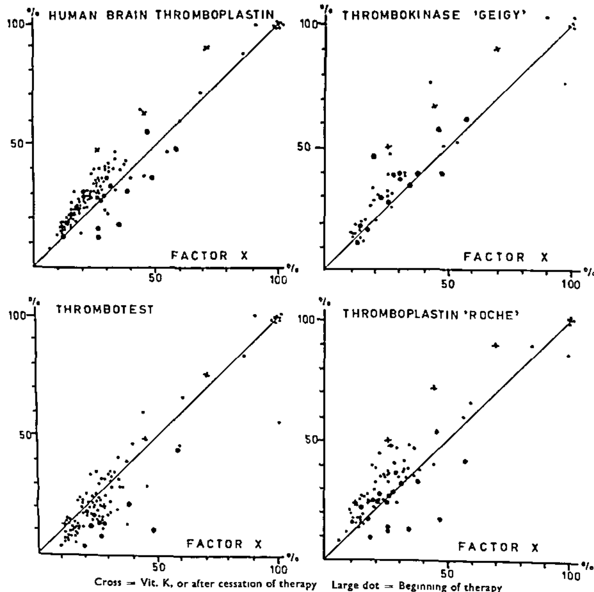
Figure I

the beginning of therapy, Thrombokinase Geigy gives higher values at this time than human brain thromboplastin, which is very sensitive to factor VII. The same was seen with Thrombokinase [illegible]

in both methods. For out-patients it is of great advantage if capillary blood can be used. We used the method described by Fiechter (2) with human brain thromboplastin and Thromboplastin Roche. This method has the disadvantage that larger volumes of capillary blood are needed. Quite good results are obtained by this method, but with Thrombokinase Geigy and Thrombotest much more reproducible results are obtained, especially within [illegible]

showed absolutely normal Thrombotest values.

PLASMA *Figure 2*



Cross = Vit. K, or after cessation of therapy Large dot = Beginning of therapy

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W. ZOLLINGER — Krankenhaus, Neumunster, Zurich
With the technical assistance of Miss L. Muller

The methods used were: Quick's test, whole blood clotting time, recalcification time, thrombelastography and the heparin tolerance test. Many authors believe that it is safe to use Quick's method alone. Others believe in simultaneous use of the methods mentioned.

The reliability and reproducibility of any particular method depend on several important factors:

1—The techniques have to be simple, so that in any laboratory the same results can be obtained by different workers.

2—The interpretation of the results should be by an experienced physician taking into consideration the anticoagulant used, whether long or short acting.

3—The greatest problem is the choice of thromboplastin.

The ideal thromboplastin should fulfil the following criteria:

(a) Simplicity in use.
(b) Sensitivity to all factors depressed by the anticoagulant.
(c) Insensitivity to factors not influenced
(d) Reproducible results by different workers at different laboratories.
(e) Same results with plasma, decalcified whole venous or capillary blood.
(f) Should not give too long a clotting time.
(g) Moderate cost.

It should be available anywhere anticoagulant therapy is practised. Such a preparation can be of animal or human origin.

We studied the commercial preparations [illegible]

[illegible] firms.

Different thromboplastins were first compared using plasma. There was a good relation between Quick's method and the Thrombotest, especially within the therapeutic range. The mean value by Quick's method is about 10% *higher than by* Thrombotest, because different diluents are used for the calibration curves, for Thrombotest Seitz-filtered plasma and for Quick's method Michaelis buffer.

[illegible] obtained when Thrombo-kinase [illegible]
When [illegible]
with [illegible]
obtai[illegible]
Geigy [illegible]
VII [illegible]

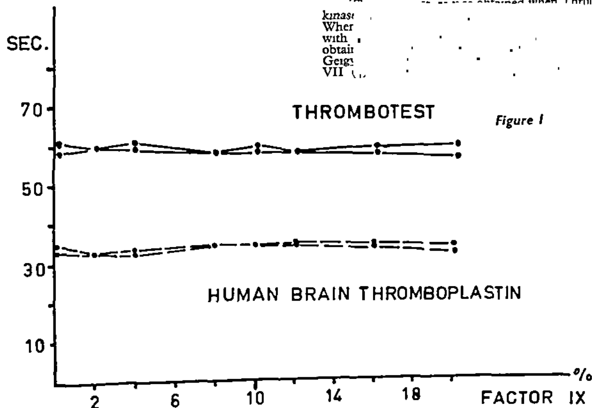
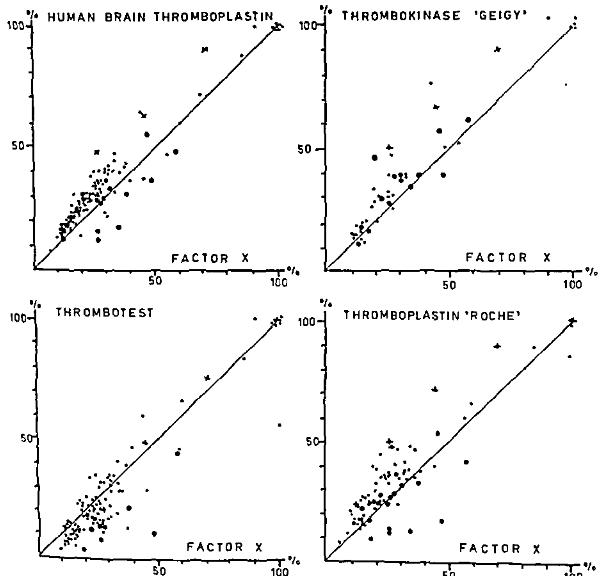


Figure I

the beginning of therapy, Thrombokinase Geigy gives higher values at this time than human brain thromboplastin, which is very sensitive to factor

in both methods. For out-patients it is of great advantage if capillary blood can be used. We used the method described by Fiechter (2) with human brain thromboplastin and Thromboplastin Roche. This method has the disadvantage that larger volumes of capillary blood are needed. Quite good results are obtained by this method, but with Thrombokinase Geigy and Thrombotest much more reproducible results are obtained, especially within the therapeutic range.

The chief property of a thromboplastin is its sensitivity to variations in any one of the clotting factors depressed. Thrombotest is claimed to be sensitive to the variations in factor IX activity. Several severe cases of hæmophilia B in Switzerland showed absolutely normal Thrombotest values



Cross = Vit K, or after cessation of therapy. Large dot = Beginning of therapy.

Comparative Study Of Control Methods For Anticoagulant Therapy

W. ZOLLINGER — Krankenhaus, Neumunster, Zurich
With the technical assistance of Miss L. Muller

The methods used were: Quick's test, whole blood clotting time, recalcification time, thrombelastography and the heparin tolerance test. Many authors believe that it is safe to use Quick's method alone. Others believe in simultaneous use of the methods mentioned.

The reliability and reproducibility of any particular method depend on several important factors:

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used, whether long or short acting.

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The ideal thromboplastin should fulfil the following criteria:

(a) Simplicity in use
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(d) Reproducible results by different workers at different laboratories.
(e) Same results with plasma, decalcified whole venous or capillary blood.
(f) Should not give too long a clotting time.
(g) Moderate cost.

It should be available anywhere anticoagulant therapy is practised. Such a preparation can be of

over other methods by being sensitive to factor IX as well as prothrombin, factors VII and X (5). Tests were strictly carried out as instructed by the various firms.

Different thromboplastins were first compared using plasma. There was a good relation between Quick's method and the Thrombotest, especially within the therapeutic range. The mean value by Quick's method is about 10% higher than by Thrombotest, because different diluents are used for the calibration curves, for Thrombotest Seitz-filtered plasma and for Quick's method Michaelis buffer.

The same picture was obtained when Thrombokinase Geigy was compared with Thrombotest. When human brain thromboplastin was compared with Thrombokinase Geigy a good correlation was obtained in the therapeutic range. Thrombokinase Geigy is rather insensitive to variations in factor VII (4) Since factor VII is rapidly depressed at

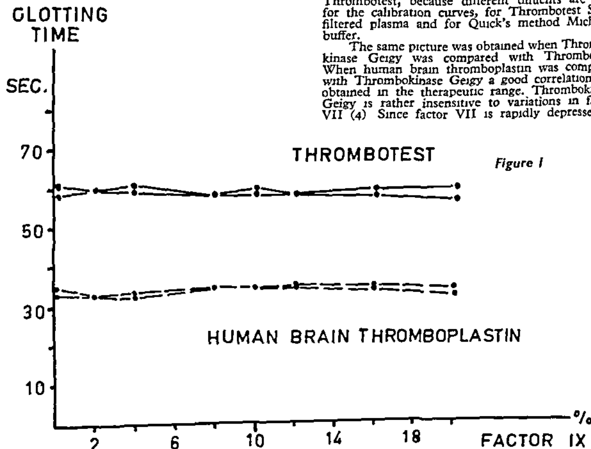


Figure I

ment factor X values are about 8% lower than those obtained by Quick's method. Different mixtures of congenital factor X-deficient plasma with normal plasma showed a similarly good sensitivity to factor X for human brain thromboplastin and Thrombokinase Geigy.

The same investigations as done on factor IX were carried out on a pure congenital factor VII-

Thrombotest shows the fastest decrease in the

These differences appear only at the beginning of therapy. Later the results are parallel.

We used the four thromboplastins in assaying factor II and factor VII in our specific one-stage

Conclusions :

1—The simplest methods are Thrombotest and Thrombokinase Geigy.

2—Factor IX activity is not detectable by any of the four methods.

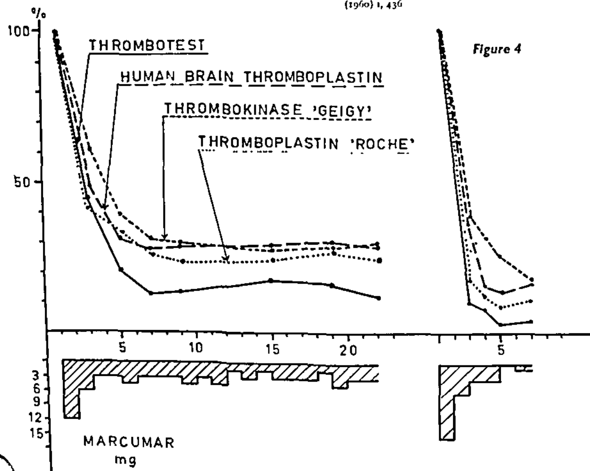
Geigy.

5—All four methods take up the same time.

6—Human brain thromboplastin, Thromboplastin Roche and Thrombokinase Geigy cost much less than Thrombotest. We hope that the cost of the Thrombotest reagent can be reduced, because it is too expensive for routine work, even if half of the original amount advised is used.

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Therefore we studied the sensitivity of Thrombotest to different factor IX concentrations. The results are shown in Fig. 1. Normal plasma was mixed with that of severe hæmophilia B in different proportions. The mixtures were diluted 1:5 to obtain plasma clotting factor levels comparable to those in anticoagulant therapy. The curves from Thrombotest and Quick's method *are identical. Therefore* Thrombotest is not sensitive to factor IX. Patients with low factor IX activity during anticoagulant therapy did not show especially low Thrombotest values as compared with Quick's method.

Fig. 2 shows results with the four different thromboplastins plotted against the factor X values in the same samples. Factor X acts both in the intrinsic and extrinsic systems. When a method is sensitive to factor X it is sensitive to (at least) one factor of the intrinsic system. Of the four preparations
correla
at the
more s… treat-

Figure 3

CLOTTING TIME SEC.
80
60
40
20
THROMBOTEST
HUMAN BRAIN THROMBOPLASTIN
THROMBOKINASE 'GEIGY'
THROMBOPLASTIN 'ROCHE'
2
6
10
14
18
%
FACTOR VII

ment factor X values are about 8% lower than those obtained by Quick's method. Different mixtures of congenital factor X-deficient plasma with normal plasma showed a similarly good sensitivity to factor X for human brain thromboplastin and Thrombokinase Geigy.

The same investigations as done on factor IX were carried out on a pure congenital factor VII-

Thrombotest shows the fastest decrease in the clotting system because it is more sensitive to factor VII, whereas Thrombokinase Geigy shows the slowest. The results with human brain thromboplastin and Thromboplastin Roche lie between. These differences appear only at the beginning of therapy. Later the results are parallel.

We used the four thromboplastins in assaying factor II and factor VII in our specific one-stage methods (3). The only variation was the change of

Conclusions :

1—The simplest methods are Thrombotest and Thrombokinase Geigy.

2—Factor IX activity is not detectable by any of the four methods.

3—It is important to have a central department to control and standardise the technique used in *different laboratories. This is well organised in* Switzerland by Geigy.

4—All four thromboplastins give good results with capillary blood, the most reliable for capillary blood being Thrombotest and Thrombokinase Geigy.

5—All four methods take up the same time.

6—Human brain thromboplastin, Thromboplastin Roche and Thrombokinase Geigy cost much less than Thrombotest. We hope that the cost of the Thrombotest reagent can be reduced, because *it is too expensive for routine work, even if half of* the original amount advised is used.

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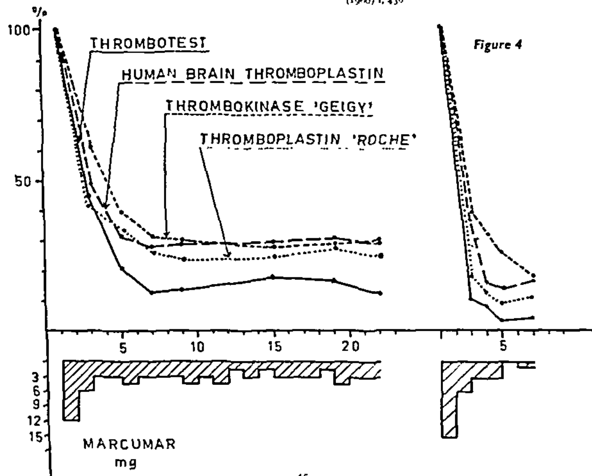


Figure 4

The Choice Of Test For Control

J. M. MATTHEWS, R. SHULMAN, A. TUXFORD and W. WALKER—

Department of Pharmacology and Therapeutics, Queen's College, University of St Andrews, and Clinical Investigation Unit, Maryfield Hospital, Dundee.

Most laboratories in Great Britain still use the

judgment are more important than the marginal advantages conferred by newer techniques It could be said that for twelve years the Quick test has worked well in hundreds of laboratories and in hundreds of thousands of patients.

Our own experience with the Quick test for control, *obtained in a relatively small hospital, is* indicated in Figs. 1 and 2. Of the two deaths from hæmorrhage only one could be ascribed exclusively

A DECADE OF ANTICOAGULANT THERAPY

MARYFIELD HOSPITAL, DUNDEE

	1950-59	1957-59
Number treated	1580	617
Serious hæmorrhage	5	2
(Non-fatal)	(0.32%)	(0.32%)
Fatal hæmorrhage	2	None
	(0.12%)	

Figure 1

to anticoagulant therapy; the other was from cerebral hæmorrhage in a very ill patient with a recent cerebral infarction Two of the five serious

the two

y of the

n-rate in

phlebothrombosis and pulmonary embolism in treated patients (Fig. 2). All three of the patients who died were very ill before treatment was started, and two were over seventy and had pre-existing cardiac and cerebral disease. On the other hand,

There is little doubt that a test possessing greater sensitivity and giving a better warning of likely hæmorrhage would permit more effective control. This has become all the more important with the increasing use of anticoagulants in arterial, especially coronary artery, disease, in which the beneficial effect has been harder to establish and in which the maintenance of the most intensive therapy compatible with safety may be relatively more important.

The theoretical and potential advantages of Thrombotest are obvious. Its sensitivity to all four factors depressed by coumarin drugs, under the conditions of

effective treat ety,

while the us able

activity of th trol

Certain difficulties, however, were encountered

great technical advance that Thrombotest has made.

Further experience has eliminated the difficulties *we found in our first trial (Matthews and* Walker, 1959). The chief difficulty with capillary blood was the large volume required, 0.1 ml . not only did some of our patients declare their preference for a venepuncture, but a free flow of

finger Moreover, the present cost of the reagent

VENOUS THROMBOSIS AND PULMONARY EMBOLISM

MARYFIELD HOSPITAL, DUNDEE, 1957-59

Deep Venous thrombosis	136 cases
	(F 104, M 32)
Pulmonary embolism	45 cases
	(F 35, M. 10)
Deaths	3 (6.6%)

Figure 2

stored in siliconed or Lusteroid tubes. Although for [illegible]

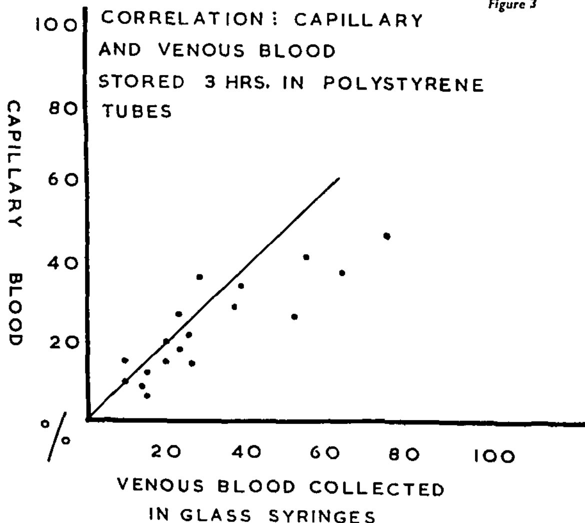
[illegible]

the cost is halved, and for the control of an out-patient would be no greater than the cost of Clinitest tablets for urine-testing in diabetics. The results correlate well with those obtained with 0·5 ml. of reagent.

With regard to the changes in stored venous blood there is no doubt that some silicones and some plastic tubes are more effective in preventing contact activation than others, and that the collection of blood in a glass syringe, unless it contains citrate, may sometimes cause activation or other changes even when the blood is stored in plastic tubes (Fig. 3) We have found three methods effective in preventing such changes: (1) as suggested by Owren, the blood is measured into a syringe already containing the appropriate volume of citrate, mixed and [illegible]

[illegible]

shows the good correspondence between the capillary test and delayed tests on stored venous blood collected by the second method. With any other method quite large variations in the coagulation activity of stored venous blood have been found quite frequently, and although at the end of 24 hours such



variations in or near the therapeutic range would [illegible]

method is preferable to measuring the volumes in the syringe, since this is more prone to inaccuracy and rather less effective in preventing activation. [illegible] sterilisation [illegible] best method [illegible] blood from [illegible]

[illegible]

fessor I. G. W. Hill we had the opportunity of studying a patient who bled on a day when his Quick test was at the upper limit of the therapeutic range. The P and P result was just below and the

[illegible]

Our conclusion is that routine laboratories should use Thrombotest with capillary blood for control of the increasing numbers of patients on long-term outpatient therapy. This was the main purpose for which the test was designed, and for which its advantages are most fully exploited Any increased cost of reagent will be offset by the saving [illegible]

[illegible] with the precautions mentioned may be used Otherwise the Quick test will continue to be used for inpatients but unless local difficulties are great, Thrombotest is preferable. For postal samples Thrombotest on blood collected in the manner

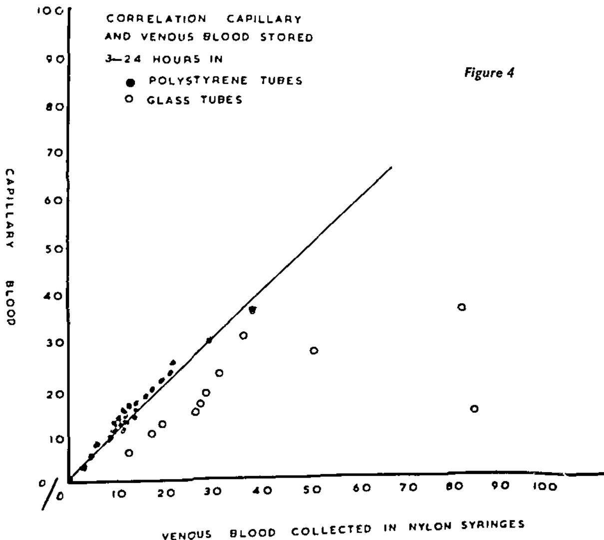
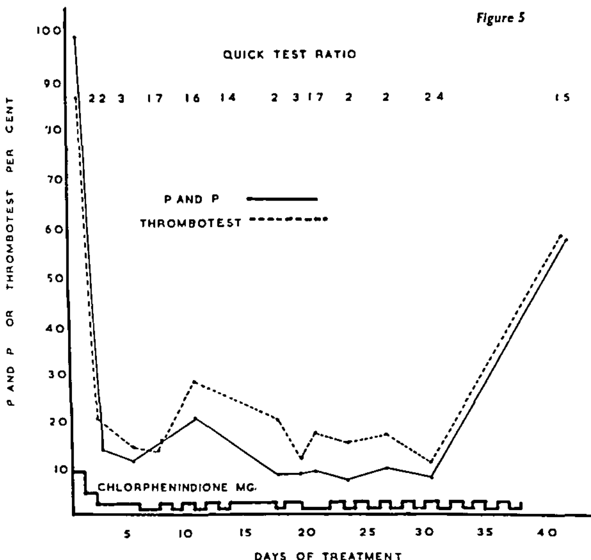


Figure 4



described is probably preferable to the Quick test. Certain laboratories with special facilities may prefer to continue with other techniques such as the P and P method or (for in-patients) thromboelastography.

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The chlorphenindione used was supplied through the courtesy of Geigy, Ltd, Manchester.

DISCUSSION

Dr BIGGS — Since the one real danger of anticoagulant therapy is hæmorrhage, the main duty of those controlling therapy is to ensure freedom from hæmorrhage. There are two main problems. One is the organisation of the service, and much could be done to improve this. The exact nature of the service will depend on local custom and personnel, but, in relation to these, serious attention should be paid to co-ordination of laboratory and clinical work. The second problem is the choice of technique. Many techniques have been used; some [illegible]

[illegible] to use in small laboratories The other, the Thrombotest, has most of the advantages of the P and P method, and is based on the use of a standardised commercial reagent I think that this Thrombotest method is the best for general use

Dr BARHAM CARTER — I have found certain difficulties in the organisation of an anticoagulant clinic The question of clinical responsibility arises—should the pathologist accept this as part of clinical pathology, or the physician, or, perhaps best of all, both equally?

I have found recurrent cerebrovascular disease difficult owing to confusional states and the difficulty of persuading relatives to accept responsibility for the tablets

I have found Quick's one-stage prothrombin time unreliable on many occasions, and think that *no price is too high for increased reliability.*

I wonder whether the results of all the tests could be expressed in time rather than percentages, but perhaps this would not give an accurate answer

Professor OWREN — Zollinger's finding that [illegible]

If the extrinsic system is blocked, as in congenital factor VII deficiency, the Thrombotest method becomes a test only for the intrinsic system. The [illegible]

of a normal standard plasma, the upper curve for a plasma totally deficient in factor IX. The difference in times illustrates the sensitivity to lack of factor IX. The sensitivity is low at 100% (undiluted plasma) because the normal extrinsic system at this concentration gives a rather short clotting time. Thrombotest, therefore, [illegible]

clinicians are asking for as a warning against a bleeding tendency during anticoagulant therapy, caused by a disproportionate reduction of factor IX.

The intrinsic clotting system is very [illegible] control plasma must be assured

Zollinger's results may be caused either by differences in technique or possibly by different defects in the plasma of his patients and ours. It is important to clarify the cause of these divergencies

[illegible] by preparing correct correlation graphs for the preparations used, and Zollinger has shown that good results can be obtained in expert hands The problem is, however, to have a simple test which gives reliable results in less experienced hands, and for this the Thrombotest method was devised.

[illegible] and meas[illegible] for I[illegible] We always recommend that technicians or doctors *unfamiliar with coagulation tests should have practical* instruction before undertaking anticoagulant therapy and control. Many visitors have come to us for this instruction, which also includes the technique of administration of anticoagulants and organisation

When venous blood has to be used, I would recommend the method advocated by Walker, to collect the blood directly from the needle into a plastic tube containing anticoagulant and marked for the correct amount of blood. We have found this most satisfactory.

The answer to Barham Carter's question, whether the pathologist or clinician should do the test and guide the dosage will depend on local circumstances. Our experience seems to show that the best results are obtained when both testing and dosage are done by the same person.

Results should always be given as percentages read from a correlation graph prepared for the method and thromboplastin used: serial dilutions

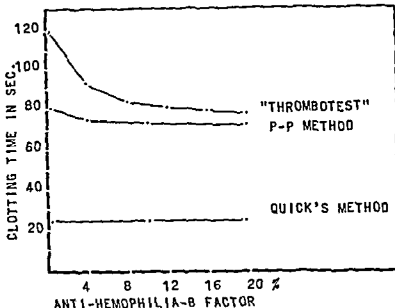


Figure 1—Sensitivity of Thrombotest to disproportionately excessive reductions of Factor IX. Plasma from a patient *with* hæmophilia B (Christmas disease) was mixed with normal plasma *in various* proportions. The mixtures were assayed after dilution 1/5 with adsorbed human plasma in order to imitate conditions during anticoagulant therapy. The concentrations of prothrombin, Factor VII and Factor X, therefore, is 20% in all samples tested.

of a normal plasma are tested, adsorbed plasma being used as a diluent. This is the only way to compare results from different methods and thromboplastins. " Prothrombin " times given in seconds are misleading even if the time for " normal " plasma is also given, because there is no correlation between prothrombin ratio and the actual clotting activity A ratio of 2 (or index of 50, twice the normal time)

[illegible]

be lowered as soon as increased production makes this possible.

Dr SAMAMA — We used Quick's test with human brain thromboplastin and Owren's Thrombotest in the last year We often use thromboelastography as well in parallel Our impression is that global coagulability is sometimes a better measure of the efficiency and safety of treatment. Two patients, for instance, may both give a Quick result of 10% but the TEG be very different, indicating a high probability of bleeding in the one and a very remote probability of bleeding in the other. *Naturally*, concurrent control by TEG and Thrombotest is very expensive.

Dr HARTERT — I should mention that thromboelastography, being *very sensitive to changes* occurring in stored blood, is not suitable for postal samples.

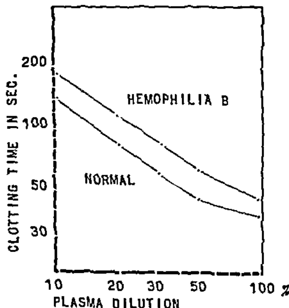


Figure 2—Dilution curves (correlation curves) for normal plasma and hæmophilia B plasma (Christmas disease plasma)

failure of Thrombokinase Geigy to measure the decrease in factor VII is taken into account, it works as well as Thrombotest.

The differences found in the factor IX sensitivity of Thrombotest are very interesting, and the reasons obscure. We carried out our investigations several times with different and severe cases of Christmas disease, with the same result. Dr Loeliger has had the same experience (personal communication).

Dr WALKER — Should not the calibration curve for Thrombotest be extended below 10% to give a better indication of the degree of excessive reduction in coagulability?

Professor OWREN — Yes, this is being done.

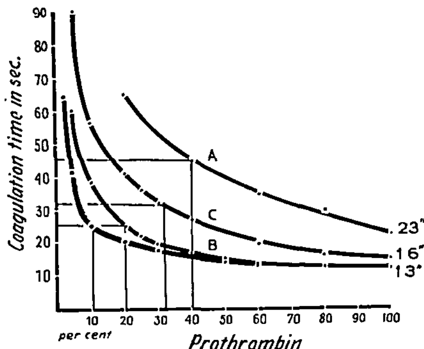
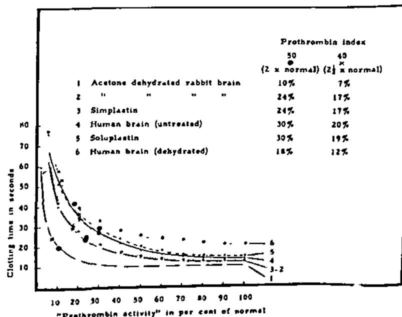


Figure 3—Correlation graphs for Quick's prothrombin time test using different thromboplastins. The curves are prepared by serial dilutions of a normal standard plasma in adsorbed plasma

Figure 4—Correlation graphs for different thromboplastins prepared as in Figure 3.



Anticoagulant Therapy In Myocardial Infarction

R. B. HUNTER—Department of Pharmacology and Therapeutics,
Queen's College, Dundee

Pioneer work on the use of anticoagulants in [illegible] engendered much heat, controversy and discord.

[illegible] one series with another.

During the earlier years of coumarin treatment, [illegible] and therefore the comparisons were of no value.

[illegible] in hospital was required.

We learned that reliable information is only likely to result from scientific investigation, that the numbers of patients in the controlled trials must be large and that meticulous attention must be paid to laboratory technique. This experience over the past fifteen years has shown, more than any other, that the days of opinion and impression in medicine have gone and advances depend on serious scientific study. In addition, close collaboration between physicians and laboratory workers is required to get the answer.

Moreover, we did not know what caused myocardial infarction Was it always, in fact, the result of coronary thrombosis? We did not know to what degree the clinical syndrome was a simple picture due to infarcted cardiac muscle or a complicated picture of which the myocardial infarction was only a part.

When the strictly controlled trials of treatment in acute myocardial infarction were first reported, the clear finding was that the incidence of thrombo-embolic complications was reduced, and because the drug used had been shown to be specific for other conditions of thrombo-embolism the results suggested that thrombo-embolism played a more important part in the mortality of myocardial infarction than had previously been thought; that many of [illegible] occurred [illegible] clinically [illegible] pulmonar [illegible] disease a [illegible] suggest that anticoagulants produced benefit in this way. There was no clear evidence, though sometimes a suggestion, that anticoagulants prevented a further myocardial infarction. What, in fact, a physician was saying, therefore, when he said he could select patients for treatment was that he could foretell which cases were likely to develop thrombo-embolic complications.

Thus mortality and the incidence of thrombo-embolism were reduced and on the basis of this alone the anticoagulant therapy of acute myocardial infarction was justified. At the same time one had to [illegible]

Today more is known about acute myocardial [illegible]

No one knows the therapeutically desirable action of anticoagulants and the criterion for the introduction of new drugs has been duration of action, giving some advantage in administration, or consistency of action. To find out what is the important therapeutic effect and to embark on a chemical adventure to try to improve that effect is a most urgent matter, because anticoagulants of the coumarin type are by no means an ideal treatment.

Professor Owren has now made the control of therapy safe and simple and capable of being con- [illegible] with safety to all who need it.

Almost everyone will now accept that in acute myocardial infarction the only patients that should be denied it are those with some clear contra-indication When the question arose as to whether long-term anticoagulant therapy might also give benefit, it was natural for physicians at this stage in our knowledge to choose serious and sometimes tragic cases, particularly in the young, for long-term treatment, often therefore cases in which serious damage to the heart and the vascular system had already occurred, and again a confused situation was created. Cases were published which appeared to benefit and others which did not.

[illegible]

complications and lower the mortality rate though there was no clear evidence that recurrence of myocardial infarction was prevented. If, therefore, one was considering the trial of anticoagulant therapy to prevent further myocardial infarction in patients who had recovered from an acute episode

presumably because further thrombo-embolic complications would be prevented.

trials completed in the last few years

Since one must accept this evidence for certain age groups, particularly men under the age of 55, the question arises whether this is sufficient evidence to say that in any disease of the vascular system which is known to be complicated by myocardial infarction the patients should have anticoagulant therapy. I believe that this is so and that, since patients with angina of effort get myocardial infarction, this in itself is sufficient justification for putting these patients on this form of treatment It

sider, on these grounds, whether we are ethically justified in denying cases of angina of effort anticoagulant therapy. Similarly, other diseases of the heart and vascular system, known to be complicated by thrombo-embolism and myocardial infarction, should also be the subject of long-term treatment

to be greater in men under the age of 55. Under the age of 55 men suffered reinfarction at one-fifth of the rate of the control group and above the age of 55 at one-half the rate of the control group. An important point is that the difference between the treated groups and the control groups is unlikely to have arisen by chance, but the difference between the age groups above 55 and under 55 may well be fortuitous. Too few cases were included in the Medical Research Council trial of females for any conclusions to be drawn.

One of the difficulties of a trial of this sort is that whereas it starts with two groups that are strictly comparable with cases randomly allocated to the high or low dosage group, after two years the groups are by no means comparable

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from the treated group at the end of two years to

risk. This is a very important study which is at present being carried out by the Medical Research Council.

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Anticoagulant Therapy In Acute Myocardial Infarction

A CLINICO-PATHOLOGICAL STUDY

R. L. RICHARDS—Southern General Hospital, Glasgow

thrombosis or its occurrence in an artery narrowed or occluded by atherosclerosis. Although we may be impressed by reports that the mortality is less in

lesions found at autopsy, and are impressed by the instances in which the treatment has been followed by rupture of the heart or some hæmorrhagic catastrophe.

MATERIAL

mortality in the three periods is about the same; roughly one-third of the patients die during their hospital stay. This figure has remained remarkably constant during the 11 years It was our policy

Heparin was given intravenously for the first 48 to

RESULTS

It is improbable that anticoagulant therapy would influence the pathological findings in patients dying within 24 hours of admission. In this group there were three instances of rupture of the heart and one of hemopericardium without rupture.

The percentage of autopsies on the fatal cases is much the same in the other two groups. There are more males than females in the anticoagulant group, whereas the smaller non-anticoagulant group comprises an equal number. The mean age at death in the two groups is similar. As regards duration of

death are similar. In both groups most deaths were due either to myocardial infarction or to cardiac

anticoagulant group there were four deaths due to this cause.

Particular attention was paid to the incidence of thrombus within the heart, pulmonary infarction, systematic infarction, cardiac rupture and any major hæmorrhage. The presence or absence of a recent thrombus in the coronary arteries was also noted. Coronary thrombosis often may follow rather than precede the cardiac infarction (Branwood and Montgomery 1956), and it seemed desirable to find

Table I

POST-MORTEM FINDINGS

	Anti-Coagulant Group Number	%	Non-Anti-Coagulant Group Number	%
Autopsies	52	100	25	100
Thrombo-embolic lesions				
Mural thrombus	13	25	15	60
Recent thrombus in coronary artery	15	29	14	56
Pulmonary infarction	3	6	7	28
Systematic infarction	6	11.5	5	20
Cardiac rupture	5	10	0	0
Hæmorrhage	8	15	0	0

out whether the incidence of recent thrombi in the coronary arteries was influenced by anticoagulants. Unfortunately, the veins of the legs were not routinely examined, and, therefore, no satisfactory data on the incidence of venous thrombosis can be given.

There is a lower incidence of all the

group and five instances of cardiac rupture. There were no such lesions in the non-anticoagulant group.

Because of the few autopsies in the non-anticoagulant group, it seemed desirable to see whether the results could be regarded as representative. A similar analysis of the 24 autopsied fatal cases in McCluskie and Seaton's non-anticoagulant group (1959) give results very like Table 1.

In my own series, one can consider the relationship of the thrombo-embolic lesions in the anticoagulant group to the anticoagulant protection given These lesions occurred in 30 patients. Of these [illegible]

were found in cases where the control was inadequate. The most significant finding is that pulmonary infarction was found in only one instance in which the patient was adequately protected

Table 2

CARDIAC RUPTURES AND HÆMORRHAGES

LESION	CONTROL
Cardiac rupture	*
,,	‖
,,	†
,,	†
Septal rupture	*
Hæmorrhagic pericarditis, lungs and pleural effusions	*
Hæmorrhagic lungs	‡
,,	*
,,	†
Cerebral hæmorrhage	†
Melæna	*
Renal hæmorrhage	†
Hæmorrhage into psoas	†

* = *Satisfactory control*
† = *Control unsatisfactory prothrombin time too low*
‖ = *One excessive prothrombin time.*
‡ = *More than one excessive prothrombin time*

Similarly, the relationship of the cardiac ruptures and the hæmorrhages to the therapy can be considered (*Table 2*). Most occurred during the first ten days of treatment, but some later. There is no evidence [illegible]

interesting patients were those who had hæmorrhagic lesions in the lungs. All four had the clinical and radiological picture of pulmonary infarction, but at autopsy they had hæmorrhagic, oedematous lungs [illegible] rce for [illegible], these [illegible] cases of [illegible]

Comparison with previous studies [illegible]

incidence of mural thrombus in the anticoagulant group is lower than that recorded in any, and of pulmonary and systemic infarction than in most untreated series reported This increases the probability that the low incidence is due to a genuine reduction brought about by the therapy.

Probably no conclusions can be based on the data regarding the incidence of recent thrombi in the coronary arteries. Although such thrombi were [illegible]

This study does not help to clarify the relationship of rupture of the heart to anticoagulant therapy. Although there were five instances of cardiac rupture in the anticoagulant group and none in the non-anticoagulant group, there were four cases of rupture in the untreated patients who died within twenty-four hours of admission The other hæmorrhagic lesions, however, represent a hazard of anticoagulant therapy, and major hæmorrhagic lesions occur even where the dosage has not been excessive. It is important to realise that hæmorrhagic lesions in the lungs may present a picture clinically similar to pulmonary infarction

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Anticoagulant Therapy In Peripheral Vascular Disease

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In peripheral vascular disease there is little argument over the use of anticoagulant drugs in the acute phase of vascular occlusion. The indications for long-term treatment are, however, much less clear. Most of the patients have atherosclerosis, with thromboangeitis obliterans next in frequency. In the latter there is threat to limb, in the former to life.

In the atherosclerotic group, more than 95% of the patients have intermittent claudication. *The fate of these is not completely known,* although subject to various studies with many disparate conclusions. Thus Hines and Barker (1940) and Spaulding (1956) followed groups of just over

ATHEROSCLEROTIC GROUP				
Analysis of 58 Cases (5 females, 53 males).				
Age. Under 40	41-50	51-60	Over 60 years	
4	9	30	15	

Table 1

colleague Dr. Richards who is doing most of the work of this investigation. The original five year survey concerned 58 patients with atherosclerosis and minimal or no nutritional changes in the legs who first attended with intermittent claudication.

The sex, age and distribution of the disease ... l 2. 17 of the ... n 13, the age ... and 15 had ... ad a diastolic

The changes which occurred in the 41 patients who *survived the five-year period are listed in Table* 4. Of these 17 had angina of effort and 13 had myocardial infarcation, 2 had congestive heart failure, 3 had cerebral vascular incidents with neurological sequelæ; 13 had a diastolic blood pressure over 100 mm Hg. 31 survivors had evidence of generalised vascular disease and in only 10 was the disease apparently still confined to the lower limbs

From these results there is a good case for long-term anticoagulant treatment in such patients, particularly those over 50 years of age with bilateral

TYPE OF DISEASE	Unilateral	Bilateral	Total
High occlusion	2	2	4
Femoro-popliteal	12	29	41
Distal	2	2	4
Asymmetrical	-	9	9
	16	42	58

Table 2

atheromatous disease of the leg vessels By this means of preventing secondary thrombotic occlusion, the prognosis might be improved even in the presence of the underlying continuing atheroma.

32 patients were placed on long-term phenindione treatment. These are in two groups. The first 16 showed *only atheroma with occlusion at the* femoro-popliteal area. Endarterectomy was performed with heparin as the anticoagulant in the operative and immediate post-operative phase. Thereafter phenindione was given for periods up to one year. The second group consists of 16 consecutive patients with severe atherosclerosis and the worst prognosis based on the criteria already mentioned:—bilateral involvement, age over 50, bad family history and evidence of generalised disease.

From the first group, it was hoped to answer the question whether or not ...

Table 3

MORTALITY	
17 patients died during follow up period (29%)	
CAUSE OF DEATH	
Myocardial infarction	7
Cerebrovascular accident	3
Congestive heart failure	2
Diabetes, tuberculosis and gangrene	1
Carcinoma pancreas	1
Perforation of peptic ulcer	1
Unknown	2

ary thrombosis and advancing atheromatous degeneration in the production of arterial occlusion. The drug has been given for periods ranging from 6 months to 2½ years (Table 5).

It appears that phenindione prevents thrombosis in a medium-sized artery devoid of normal intima

SURVIVORS 41 cases		
Unchanged or improved		27
Worse		14
Unilateral ⟶ bilateral	3	
Acute arterial occlusion	3	
Definite deterioration	5	
Amputation	3	
		41

Table 4

but the important change is not so much secondary thrombosis as unrelenting encroachment on the arterial lumen by the atheromatous degeneration.

to 10 years. Their ages range from 18 to 35 years,

(Hutchison).

Table 5

long term anticoagulant therapy (phenindion

Group	Number	Age	Complications
Endarterectomy for isolated lesion at femoro-popliteal area	16	39-59	Reocclusion at 2/12 1 Myocardial infarct death at 8/12 1
Severe generalized atherosclerosis	11	52-69	Amputation for gangrene (Bilateral in 3) 4 Myocardial infarct, cerebral thrombosis, myocardial infarct - death 1

Ten cases fulfil the diagnostic requirements. In 5 the presentation was distal ischæmia and toe skin breakdown. In these lumbar sympathectomy with local removal of dead tissue was carried out. Thereafter treatment with phenindione was begun and continued until healing occurred. Should

is slowly stopped over a period of three weeks.

to tide the patients over to middle life when the disease is self-limiting.

In acute occlusive processes heparin is the undoubted drug of choice. This group includes

The efficacy of heparin in acute vascular occlusion is also underlined in six patients with phlegmasia cœrulea dolens in whom the drug given intravenously with elevation of the limb was successful in re-establishing venous drainage of the leg. In three of these cases, phenindione had been given previously for deep vein thrombosis and it would appear that the further venous occlusion had occurred in spite of this. In a further five cases seen at the stage of venous gangrene, phenindione had

to survive since the actual tissue loss is frequently less than initially appears likely.

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Prophylaxis By Anticoagulants (Coumadin) In Surgical Disorders

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Coumadin (Warfarin) was used for the prophylaxis of thrombo-embolism in orthopædic operations on 31 cases—19 women and 12 men.

Their age varied from 20 to 90—21 being over 60. The average length of treatment was 57 days, most being treated for 20 to 60 days. The patients were immobilised throughout.

Coumadin was given in a single daily dose.

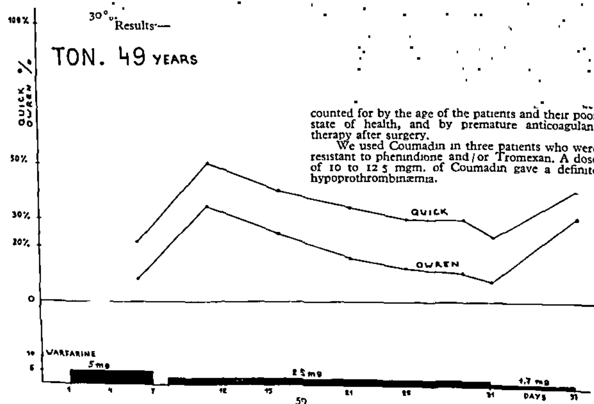
The initial dose was 10 mgm. for the first two days in younger patients, 5 mgm. in patients older than 50, and 2.5 mgm. in poor risk patients or in patients older than 60. The average maintenance

complex VII (Stuart factor and proconvertin) assay. The last three investigations were not carried out routinely.

Control tests were carried out three times a week in the first week, twice a week in the following weeks, and once a week when the treatment extended over 40 days.

30%.

Results—



A stable therapeutic level was reached within 5 to 8 days, and was easily maintained in 22 patients but less easily in 6, who were elderly as well as poor risk.

periods were exceptional.

In parallel with Quick's test and heparin tolerance we did 180 Thrombotests. We noted the simplicity of the method, its reproducibility, and its extreme sensitivity.

The results accord with those of Quick's test (Fig. 1), but the values of Thrombotest were always lower.

Simultaneously, we measured coagulability using the thromboelastogram and the heparin tolerance test. We feel that these techniques more accurately anticipate the risks of hæmorrhage and thrombo-embolism. The prophylactic range of 30 to 40% (Quick) or 20% (Owren) corresponds to a moderate hypocoagulability.

We had a thrombophlebitis in one patient, owing to inadequate therapy.

In 4 cases an abnormal bleeding tendency was attributed to therapy: melæna in a patient aged 63 with an undiagnosed carcinoma of the alimentary

counted for by the age of the patients and their poor state of health, and by premature anticoagulant therapy after surgery.

We used Coumadin in three patients who were resistant to phenindione and/or Tromexan. A dose of 10 to 12.5 mgm. of Coumadin gave a definite hypoprothrombinæmia.

Prophylaxis By Anticoagulants After Gynaecological Operations

SELECTION OF PATIENTS

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Pulmonary embolism is a common cause of death after

operations, despite "early ambulation" and daily examination to detect and treat venous thrombosis as early as possible.

The difficulty is, of course, that the thrombosis is often silent and insidious. In two-thirds of the cases of

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signs we

transient to be disregarded.

Marks et al (1954) noted

Prophylactic Anticoagulation.

A trial using

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cent;

30 per

stage method was used.

While the incidence of deep vein thrombosis was the same in both groups (1 5 per cent.), there were no emboli in the 201 cases treated compared with

there was no more bleeding in treated cases than controls

Severe bleeding, requiring blood transfusion and

from hæmorrhage.

Selection of Cases for Prophylactic Anticoagulant

The trial showed that

The possibility of selecting "high risk" cases was studied in three groups of cases —first, 3,040 patients who had a major gynæcological operation between 1951 and 1954, of whom 119 developed thrombo-embolism; second, 30 patients who died of embolism after

and 195

tions be

prophyl

Many factors are thought to be associated with increased risks of thrombo-embolism, but analysis showed finally that cases could be selected for treatment on the basis of only three factors—age, body weight, and past or present "venous" complications.

Age.

Age was found to increase very considerably the risk of death from embolism, although it only increased slightly the

in the younger group.

Of the 30 cases of fatal embolism between 1937 and 1954, twenty-two were 50 years or more, five were between 45 and 49, and only three under 45.

Obesity.

The weight-height ratio was used as an index of

wh

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wit

tria. who did not develop any thrombo-embolism

Fifty per cent. of controls were of "normal" weight, compared with only 24 per cent of complicated cases. Of the 19 complicated cases of "normal" weight, only four (20 per cent.) had an embolism and none was fatal, but of the 60 obese cases, 24 (40 per cent) had an embolism and six died. Weight or height had not been measured in all cases, but of the four fatal emboli where W-H

Previous Thrombo-embolism and Varicose Veins.

Accurate information about these factors

Of the 67 cases with previous complications, ten (15 per cent.) developed complications, three times as many as in cases without previous trouble. Pulmonary embolism was more than twice as common and one case was fatal. Varicose veins were found in

almost one-third of all cases, and the incidence of venous thrombosis (6 per cent.) and of embolism (4 per cent.) was higher than in the group without varicosities (2.3 and 1.7 per cent. respectively). Moreover, of the thirty patients who died from embolism between 1937 and 1954, only three were under 45 years of age, and all three had a history of previous deep venous thrombosis.

Criteria for Selection of Cases.

If, between 1937 and 1954, prophylactic anticoagulants had been given to all women who were both 45 years or more and overweight, or who had varicose veins or a history of thrombo-embolism, and if the treatment had been effective, at least 25 of the 30 deaths from embolism would have been prevented. (Body weight was not known in the other five patients, although all were over 45 years of age.)

It was decided to introduce a policy of prophylactic anticoagulation in both units, using these criteria to select patients.

Preliminary Results in Anticoagulant Prophylaxis in Selected Cases.

This policy has been in practice since April 1957. Phenindione was given from the 1st to the 12th post-operative days and the prothrombin concentration kept at 25-30 per cent.

The results refer to pelvic floor repair. (Details are not yet available for other operations, but it is understood that no deaths from embolism have occurred.) The incidence of thrombo-embolism is much less in the 1957-1959 series (1.5 per cent.) than in 1951-1954 (4 per cent.) when there was no prophylaxis, but the striking difference between the two groups is in the number of fatal emboli, with only one death in 835 patients between 1957 and

developed in the 465 untreated cases, of whom one died of embolism. Actually, this patient should have been treated prophylactically because she had severe bilateral varicose veins.

The criteria, therefore, for selecting cases for treatment appear to be adequate and satisfactory in practice, but the decision NOT to give prophylactic anticoagulants after a major gynæcological operation should be made only after careful assessment of the patient.

Of the 370 cases treated, 53 (14 per cent.) had bleeding and this was severe, necessitating blood transfusion or further operation in 11 (3 per cent.). There were no deaths from hæmorrhage. In the untreated group, only 15 of the 465 cases (3 per cent.) had any bleeding at all.

In the 1955-1956 trial, 37 per cent. of pelvic floor repairs had bleeding following phenindione treatment, so that an incidence of 14 per cent. in this larger series shows that the risks have not proved as great as expected.

Conclusions.

The incidence and mortality of thrombo-embolism following gynæcological surgery can be greatly reduced by prophylactic anticoagulation with phenindione. Chalmers et al (1960) have recently reported similar conclusions following five

ing the use of prophylactic anticoagulants.

The policy is practical, safe and effective.

I am grateful to Professor Dugald Baird for permission to publish data obtained from investigations which were, and are being carried out in his department. I am also indebted to Dr A. Herriot, who supplied me with the details of the results of treatment in pelvic floor repair cases between 1957 and 1959

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The Role Of Anticoagulant Therapy In Cerebral Infarction

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The role of anticoagulant therapy in cerebral infarction has been a source of controversy since

ate short-term measure in acute cerebral infarction to prevent the spread of the infarcted area and so conserve its marginal viable tissue, or as a long-term treatment to prevent further cerebral episodes in patients with a history of previous and recurring cerebral infarction.

Current Views.

The results of clinical trials have so far remained as inconclusive as that of Hedenius and owing to possible dangers in treatment views have been strongly divergent.

The studies of Sibley (1957), Moyes (1957), Peterman (1959), and their colleagues showed that dicoumarol increased the hæmorrhagic tendency

(1960) have shown that the routine use of anticoagulants in unselected patients with clinical cerebral infarction not due to cerebral embolism

of fatal cerebral hæmorrhage was greater in the treated than the untreated groups. This trial, however, included some hypertensive patients.

The Series

My own experience, some of which has been previously reported (Carter 1957, 1959, 1960) ... The first is a series of patients ... ion treated from ... The second is a ... its from 1955 to ... evious attacks of ... hæmic episodes

Acute Cerebral Infarction.

... patients taken from ... mitted to
1002 ... to 1958
Ashfc ... suffering
(Tab ... onsidered
from
separately (Table 2).

Anticoagulants were given to patients with clinical cerebral embolism in 1954, unless there was blood in the cerebrospinal fluid, unless they were over 70 years of age and unless they had a persistent diastolic blood pressure over 120 mm.Hg. Five patients were rejected on the above grounds. The

In the group of patients suffering from atheromatous cerebral infarction without obvious embolism

of patients for a further trial of anticoagulants on the grounds of slowness of onset and incompleteness of lesion It was soon found that speed of onset was almost impossible to measure, and patients were therefore selected who showed progression of clinical signs while in hospital or who gave a clear history of progression of symptoms immediately before admission and whose lesion was by then complete. In addition the diastolic blood pressure had to be persistently below 120 mm of Hg., the cerebrospinal fluid clear, age below 70 with few exceptions, and the history free from symptoms of peptic ulcer or hepatic disease. In the four years 1955 to 1958, 83 patients out of 277 admitted with the diagnosis of "cerebral thrombosis" satisfied the above criteria, but three had to be withdrawn as a history of visceral bleeding emerged within a few days of treatment Of the remaining 80, four were found to have been wrongly diagnosed, by chance two in each group. Three were found at autopsy

coagulants—about one in seven.

Recurrent Cerebral Infarction.

The problem of recurring cerebral ischæmia

TABLE 1

Cases of "stroke" admitted to Ashford Hospital, 1952-58, showing sex and ætiology.

No. of cases	1002
Sex:	
Male	478
Female	524
Ætiology:	
Embolism	85
Thrombosis	425
Hæmorrhage	492

TABLE 2

Cases of cerebral embolism, 1952-58.

No. of Cases	Males	Females	Average Age	Mitral Stenosis and Fibrillation	Mitral Stenosis
85	32	53	53	39	5

Fibrillation	Cardiac Infarct	Subacute Bacterial Endocarditis	Thyrotoxicosis and Fibrillation
7	26	4	4

TABLE 3

Statistical comparison of results in the treatment of cerebral embolism $P = 0.025$.

Year	Total	Patients who recovered or were improved	Patients who died or were not improved	Percentage recovering
1952	16	5	11	31
1953	18	6	12	33
Treatment—No anticoagulants				
1954	15	11	4	73
1955	9	6	3	66
1956	9	6	3	66
1957	9	6	3	66
1958	7	4	3	57
Treatment—Anticoagulants				

TABLE 4

Results of a controlled trial in patients with progressive atheromatous cerebral infarction, 1955-58

Total No. of Cases	76
Anticoagulant Therapy	
No. of cases	38
Recovered or improved	26
Not improved or died	12
No Anticoagulant Therapy	
No. of cases	38
Recovered or improved	19
Not improved or died	19

with or without infarction was investigated from 1955 to the end of 1958. The series is a very small one, as only 32 patients below the age of 70 could be found who gave a history of two or more episodes of cerebral ischæmia or cerebral infarction, and whose diastolic blood pressure was below 120 mm. of Hg. Sixteen of these patients were selected at random and put on long-term anticoagulant therapy, and their progress in regard to recurrence and survival compared with the 16 controls, with a follow-up varying from one to four years.

The recurrences have been divided into two groups. Minor recurrences are those showing transient neurological deficits followed by a return to previous disability, or by only a slight worsening as judged by the patient's ability to get about and look after himself. Major recurrences are those showing longer and more severe neurological deficits often producing permanent deterioration. In the control group three patients had no recurrence, one had minor recurrences, seven had a major recurrence and five had both. Twelve patients, therefore, out of the 16 had one or more major recurrences, and of these six died. In the treated group, six patients were free from recurrence, three had minor recurrences, three had a major recurrence and four had both. Seven patients out of the 16, therefore, had one or more major recurrences, and of these four died.

If the patients with hindbrain ischæmia are considered separately, there were four in the control series and five in the treated. Of the controls one had minor recurrences and three had major and minor recurrences, two of these dying. Of the treated [illegible]

[illegible] may be worth while (Table 6).

Mechanism of Treatment.

[illegible]

ineffective and occasionally harmful. One must also recognise that apparently adequate prolongation of the prothrombin time will not always prevent

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tment of

Dangers of Treatment.

The dangers of anticoagulant therapy are three in number. The first is bleeding into and around the infarcted brain; the second is major visceral bleeding producing systemic hypotension; and the third is the risk of treating intracranial hæmatomata with anticoagulants because of errors in diagnosis. These dangers are very real and stress the importance of strict control, and selection of patients, and above all of correct diagnosis, which may be more difficult than is commonly supposed, and even investigation by arteriography is often unhelpful. Fortunately in this series no major visceral bleeding was encountered during the last three years, but there were 17 minor hæmorrhagic episodes in nine patients. Hæmaturia accounted for seven, epistaxis

the term subacute cerebral infarction might be used to describe this type of case. It is clear from the work of Marshall and Shaw (1960) and from my own

progressive cerebral infarction and recurrent cerebral infarction subject to the usual contraindications. Although this decision is not wholly supported by my past figures, there seemed sufficient evidence to justify it

TABLE 5

Results of treatment if lesion is incomplete and if lesion is complete.

No. of Patients 76

	Anticoagulant Therapy			
	Lesion Incomplete		*Lesion Complete*	
	Group A	Group B	Group A	Group B
	17	5	9	7
Per cent.	77	23	56	44
	No Anticoagulant Therapy			
	Lesion Incomplete		*Lesion Complete*	
	Group A	Group B	Group A	Group B
	10	10	9	9
Per cent.	50	50	50	50

Group A = Recovered or improved.
Group B = *Unimproved or died.*

TABLE 6

Results of anticoagulant therapy in recurrent cerebral infarction.

TOTAL CASES—Follow-up 1 to 4 years.

CONTROL GROUP:	
No of patients	16
No recurrence	3
Minor recurrence	1
Major recurrences:	
Surviving	6
Died	6
TREATED GROUP:	
No of patients	16
No recurrence	6
Minor recurrence	3
Major recurrences:	
Surviving	3
Died	4
HIND BRAIN ISCHÆMIA.	
CONTROL GROUP:	
No of patients	4
No recurrence	None
Minor recurrence	1
Major recurrences.	
Surviving	1
Died	2
TREATED GROUPS	
No of patients	5
No recurrence	3
Minor recurrence	1
Major recurrences.	
Surviving	None
Died	1

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Anticoagulant Therapy In Cerebrovascular Disease

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The cerebral circulation is peculiarly complex and anatomically variable; with its efficient anastomotic potential across the mid-line, its pressure gradients are highly unpredictable and vascular lesions may produce their ischæmic effects at remote sites and even outwith the territory of supply of the vessel affected. Unfortunately little of the cerebral vasculature is accessible to clinical examination. We can palpate and auscultate the carotid arteries in the neck and inspect the retinal vessels; we can compare the pressures in the retinal arteries on the two sides with the ophthalmodynamometer but these are the limits of our clinical assessment. The effects of ischæmia cannot be accurately detected electrically as they can in the case of the heart muscle, and the value of contrast radiography is at present limited by its permitting examination only of the major vessels. In a recent study of eighty patients submitted to angiography following an acute "stroke," there were forty-six in whom no radiological abnormality was demonstrated (Bull, Marshall and Shaw, 1960).

The diagnosis of cerebrovascular lesions depends largely on the indirect evidence provided by the resulting neural damage; and the same neural pathways may be interrupted and the same clinical picture produced by a wide variety of pathological processes. We are particularly handicapped by an inability to distinguish with certainty between infarction and hæmorrhage, a point of obvious significance in relation to anticoagulant therapy. The presence or absence of blood in the spinal fluid, although of value, is not pathognomonic.

Besides, we cannot predict with any accuracy the probable outcome of a "stroke." In the early stages we have no means of knowing whether the functional deficit is due to destruction of neurones or to reversible impairment of discharge or conduction and hence apparently dense deficits may show rapid and unexpected recovery.

The implications of these facts are obvious for the assessment of anticoagulant therapy in cerebrovascular disease. Clearly the variability in prognosis makes controlled study obligatory. The diagnostic uncertainty is perhaps most disturbing in connection with the recurrent minor impairments of function—the so-called "transient ischæmic attacks"—for these, particularly in brain-stem territory, are readily mimicked by non-vascular ... It is probable that the conflicting experience ... some measure to ... ysical symptoms ... uishing between ... rhaps the most ... and it may prove ... use of anticoagu- ... In uncontrolled studies the significance ... plication of low numerical incidence may not be apparent but Barron and Fergusson (1959) have drawn attention to the large number of fatal cerebral hæmorrhages in the extensive literature on anticoagulant therapy in cerebrovascular disease. The possibility will be discussed later that these complications of treatment are in fact the result of misdiagnoses of the original presenting illness.

In designing our trials of anticoagulant therapy in cerebrovascular disease, started in 1957, it was decided to consider the problems as they most commonly present in general medical practice. By ... had been several highly favourable ... vascu- ... ral in- ... agnostic uncertainties ... fic subgroups represent a very small pro... cerebrovascular disease, it was felt that the value of anticoagulant treatment should be assessed in the broad spectrum of cerebrovascular cases encountered in everyday practice.

The results of two trials will be described briefly. Details of design and composition of the trials have been reported (Marshall and Shaw, 1960; Hill, Marshall and Shaw, 1960). The first trial was designed to assess the value of anticoagulant therapy in patients who had suffered an acute "stroke" believed to be due to non-embolic cerebral infarction. All patients were under 70 years of age; treatment was started as soon as possible after the onset of the attack but 72 hours was the maximum interval permitted. In addition to clinical diagnosis, all eligible patients had their spinal fluid examined, principally to confirm the absence of blood, and were submitted to cerebral angiography. Blood in the spinal fluid, severe hypertensive retinopathy or left ventricular failure excluded patients from the trial, the latter because it might demand the use ... Peptic ulceration and bleeding ... indications. ... the basis ... pairs and allotted to treatment ... Those in the treatment group were given an initial dose of 300 mgs. phenindione and, starting twelve hours after their angiogram, three doses of heparin (12,500 units) were injected intravenously at six-hourly intervals. From the second day onwards treatment was continued with phenindione, the ... thrombin time (Quick one-stage test) being ... three times the control; ... days and ... nd control ... h regard to ... iotherapy. ... the trial, twenty-six in the treat... twenty-five in the control. There were twenty-four males and twenty-seven females with approximately even distribution of the sexes to the two groups. The anatomical sites of the vascular lesions based on clinical and angiographic findings were very comparable in the two groups.

Survival or failure to survive for six weeks from the start of treatment was the criterion on which results were based and these were analysed by means of a restricted sequential procedure. We were indebted to Dr Peter Armitage for the detailed statistical design which is embodied in the sequential chart (Fig. 1). Patients were considered in pairs according to the original random allocation to treatment and control groups. If both members of a pair survived or both died no preference was indicated in respect of that pair for treatment or control and no result was plotted. If, however, members

of a pair behaved dissimilarly a preference was plotted and the direction of the arrow indicated whether it was the treated or control member who had failed to survive. Arrows pointing downwards and to the right represent the pairs in which the treated member died while the control member survived; arrows pointing upwards and to the right *indicated pairs in which it* was the treated member who survived. The horizontal axis represents the total number of preferences while the vertical axis represents the number of pairs showing preference for anticoagulation treatment minus the number showing preference for control treatment The apex of the plot thus registering a minus value (Fig. 1), indicates a trend in favour of the control treatment. Had the upper or lower boundary been reached, it

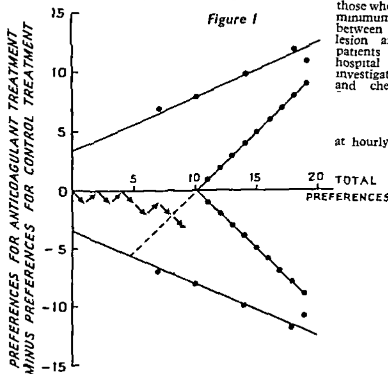
a point was reached when the upper boundary was no longer attainable and the trial was therefore stopped with no significant difference between the treatment and control groups demonstrated.

In all there were six deaths among the twenty-six patients in the treated group and three deaths among the twenty-five controls. Post mortem examinations were carried out on eight of the nine patients who died. Three of the six patients in the treatment group who died showed evidence of cerebral hæmorrhage. The possible implications of this will be discussed later, but the general conclusion drawn from this trial was that anticoagulants are not of value in acute strokes when used according to our criteria of selection and management.

The second trial was designed to assess the value of long-term anticoagulants as a prophylactic measure in patients suffering from cerebrovascular disease. The trial was designed in collaboration with Professor Bradford Hill. The precise objective was to determine whether or not the expectation of life could be increased or the incidence of further cerebrovascular accidents decreased in patients with cerebrovascular disease. Again we did not confine ourselves to any particular diagnostic subgroups but included the whole range of cases most commonly encountered in general medical practice. Patients were considered for inclusion who were under seventy years of age and in whom there was evidence of a previous neurological disturbance lasting for more than twenty-four hours and judged by the clinician caring for the patient at the time, and by ourselves subsequently, to be due to non-hæmorrhagic cerebral, carotid or vertebro-basilar arterial disease. No distinction was made between those who had made a good functional recovery and those who had been left disabled. In all cases a minimum of fourteen days had elapsed between the last acute cerebrovascular lesion and inclusion in the trial. All patients considered for the trial attended hospital as out-patients for a day for investigation and assessment. X-rays of skull and chest, electrocardiogram and renal

at hourly intervals with the patient supine. The usual contraindications to anticoagulant therapy were observed and patients were also excluded if it was felt that they would be unable or unwilling to co-operate sufficiently or to attend regularly for the necessary supervision.

Patients accepted for the trial were differentiated by sex and randomly allotted in pairs to one of two groups. The treated group (high-dosage group) received tablets containing 50 mg. phenindione, while the control group (low-dosage group) were given apparently identical tablets containing 1 mg. phenindione, an amount insufficient to affect the clotting mechanism The patients did not know to which group they belonged and both groups were managed in the same way with regular prothrombin estimations at intervals not exceeding four weeks In the high-dosage group the aim of treatment was to maintain the prothrombin time (Quick one-stage test) at a level of two to two-and-a-half times the control value



Restricted procedure for $\theta = 0.9$ with maximum number of preferences, $n = 19$.

Suppose θ is probability of preference for anticoagulants.

If $\theta = 0.5$ (null hypothesis), probability of reaching each outer boundary is 0.022 (i.e., slightly lower than the nominal 0.025).

If $\theta = 0.9$, probability of reaching upper boundary is 0.97 (i.e., slightly higher than the nominal 0.95).

(Points at which the boundaries can be reached are shown as circles.)

Many of the criteria for selection and details of management were based on the plan of the Medical Research Council trial of anticoagulant therapy in coronary thrombosis (M.R.C. 1959), to which access was kindly granted, and we gratefully acknowledge the help thus received.

One hundred and forty-two patients were admitted to the trial, 71 (47 men and 24 women) to the treatment group and 71 (47 men and 24

TABLE 1.—SUMMARY OF RESULTS.

	High Dosage Group Male	Female	Total	Low Dosage Group Male	Female	Total
Total No of Patients	47	24	71	47	24	71
Non fatal cerebrovascular accidents	3	2	5	4	—	4
Fatal cerebrovascular accidents	2	2	4	—	—	—

* One died of myocardial infarction, and one is alive and has had no further cerebrovascular accidents

group and four recurrences in three patients in the control group, a difference which is clearly not significant. However, there were four fatal cerebrovascular accidents in the treatment group and none in the control. Furthermore all of these deaths were due to hæmorrhage and that they were related to

cardial infarct, but this was not confirmed at post

observing by chance five deaths in the one group and none in the other does not quite reach the formal

cerebral hæmorrhage were, in fact, infarcts in which bleeding had been induced seems unlikely both on pathological grounds and by inference from the absence of a similar incidence of infarction in the control group It seems improbable, too, that inadequate control of therapy in our series can be held responsible since our complication rate in other respects does not compare unfavourably with other series (Table 2)

The incidence of cerebral hæmorrhage in

due to hæmorrhage than has formerly been supposed. We suspect, and there is supporting patho-

hæmorrhage, three were among the twenty-four hypertensives in the treatment group and one was from the forty-seven who were not hypertensive. These figures are not significant, but in further studies we are avoiding the use of anticoagulant treatment in hypertensive subjects.

In conclusion it must be stressed that these results need not necessarily conflict with more favourable reports of experience with anticoagulant drugs in specific cerebrovascular syndromes. We have so far attempted only to assess the value of anticoagulants in the two commonest situations in which the physician is concerned in the treatment of acute and chronic cerebrovascular disease. From our studies we believe that the general use of anticoagulant therapy in these situations is not to be recommended.

TABLE 2.—COMPLICATIONS OF TREATMENT

	High Dosage Group Male	Female	Total	Low Dosage Group Male	Female	Total
Total No of Patients	47	24	71	47	24	71
Hæmorrhage—Treatment interrupted	5	8	13	1	—	1
Hæmorrhage—Treatment abandoned	2	—	2	—	—	—
Hypersensitivity Treatment abandoned	—	1	1	—	—	—
Intercurrent Illness—Treatment abandoned	—	—	—	—	1	1

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DISCUSSION

Professor HILL — A very high proportion of the deaths in myocardial infarction occur in the first 24 hours after onset. These are in general due to causes not amenable to anticoagulant therapy:

of the disease.

I should like to refer to a use of anticoagulants not mentioned hitherto, namely their value in cutting short the ingravescent cerebral thrombosis in dehydrated infants with cyanotic congenital heart disease. A small venesection (20 ml.) and tiny doses of heparin can save some such infants from a crippling hemiplegia, which if unchecked may render later operation for the cardiac condition unjustifiable.

The experience of Mr F. R. Brown in respect of the incidence of pulmonary embolism after operation for thyrotoxicosis should be mentioned. In

xine or tri-iodothyronine pre-operatively as a prophylactic?

is probably that the number of patients is too small. A recent study in Sweden showed statistically significant effect above 60 years of age but not below.

The situation in myocardial infarction is very probably the same as in angina pectoris, the main factor influencing the therapeutic effect being the "age" of the disease prior to therapy and not the actual age of the patient.

We believe that it is not correct to consider age above 60 years as a contraindication to therapy. This would also exclude the majority of women because of the delayed onset of coronary disease in the female.

Richards found no direct relationship between

Hunter and Richards stressed the importance of an adequate depression of the "prothrombin" level in order to obtain therapeutic effect. There seems to be no doubt that many patients are undertreated because this important fact is not correctly understood. This is often caused by incorrect assessment of the therapeutic level for the actual

of the hæmorrhagic tendency. An absolutely stable level at a specified percentage cannot be achieved. The practical result, therefore, is that we should aim at a level of 15% with 10% and 25% as the lower and upper limits.

from patients on anticoagulant therapy have normal concentrations of these factors and, therefore, give shorter clotting times than those shown by the correlation curve for the corresponding concentrations of the 4 factors depressed. If adsorbed plasma is used as diluent, instead of saline, Thrombotest and Quick's test give more identical results, differences occurring when factor IX is disproportionately reduced. It must further be remembered that whenever methods are compared the same standard plasma must be used for all correlation curves.

Dr MITCHELL — I wish to stress some of the dangers inherent in statistical comparisons between various groups of patients. No investigator approaches a problem without some idea about the values and dangers of a particular treatment, and however great the care taken to avoid it, bias is inescapable. Thus, Dr Richards said that his pathologists had been struck by the incidence of various events in patients on anticoagulants; it surely would have been better thereafter for the pathologists to be unaware of the treatment of the

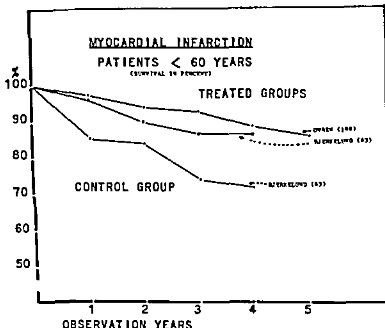
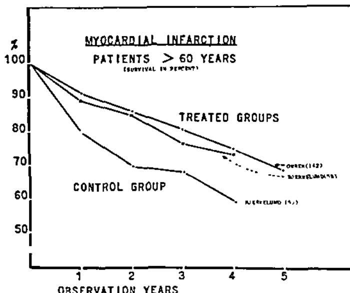


Figure 1 (left) — The effect of life-long anticoagulant therapy on mortality of survivors of acute myocardial infarction. Survival curves for patients below 60 years of age

Figure 2 (below) — Survival curves for patients above 60 years of age.



Dr Barham Carter's assessment of

correlation between the patient's own assessment of his progress and the objective assessment by exercise tolerance test.

placebo and dies because anticoagulant is

groups.

Dr SCHWARTZ — Dr Richards's figures for myocardial rupture in treated and untreated cases of myocardial infarction might be interpreted as evidence that anticoagulants predispose to rupture of the myocardium However, a rupture incidence of 10% of

anticoagulants delay the time of rupture, or that there has been some unusual selection of cases.

Dr VERSTRAETE — This study concerns 107 outpatients who have been treated for 1302 patient-months with dicoumarol derivatives Half of these patients were maintained on anticoagulant therapy for more than six months.

are very sensitive to dicoumarol derivatives (3 to 9 mg Marcoumar per week), others on the contrary develop a progressive resistance (42-54 mg. Marcoumar per week).

During the 1302 months of treatment, there were 7 relapses of thrombo-embolic phenomena. In 4 of these patients, the Quick test was above the therapeutic range 12 hours after the accident; 27 minor hæmorrhages were observed during the 39,060 days of treatment (125 years) and 3 severe hæmorrhages, one of these being fatal (cerebral bleeding).

Besides the indications for long-term anticoagulant treatment, there is an urgent need for proper evaluation of coumarin drugs combined with vasoactive substances. New and simple methods for early detection of minor bleeding tendencies should be developed as a safety measure.

to determine the value of anticoagulant treatment for the survival time in cerebral thrombosis and embolism. The only criterion was life or death.

There were in all 66 cases of cerebral thrombosis not treated with anticoagulants and a regular follow-up was obtained in 45. Nineteen patients survived the initial thrombosis for an average period of 39 months. Twenty-six patients with an average age of 63 years died after 15 months. If the first month of survival is excluded, the mean survival time was 24 months.

Of the 41 treated patients with cerebral thrombosis, no recent information was obtained in 14. Of the remaining 27, 21 (mean age 61 years) were still alive 131 months after the initial thrombosis. Only 6 patients in this group died after an average of 33 months.

Thus, 57.7% of the 45 patients with cerebral thrombosis not treated with anticoagulants died after 15 months compared with 22% of the 27 anticoagulated patients who died after 33 months.

Of 6 untreated patients with cerebral embolism who could be traced, there was an average survival time of 35 months, and 3 deaths after 2 months. Of 8 patients with cerebral embolism treated with anticoagulants 7 are still alive after 22 months and only 1 died after 5 months.

Dr MARSHALL — As regards Dr Mitchell's criticism, if the series is large enough errors in diagnosis will be distributed equally between treated and control groups. In any case, when both members of a pair die this is a "tied" pair which does not affect the sequential analysis. In our study, all deaths in fact occurred in "untied" pairs.

Dr TURNBULL — It is true that older patients often have pelvic floor repairs, but the type of

repairs.

Dr HARTERT — Concerning reinfarctions, we made a scrupulous follow-up by thrombo-elastography of a number of myocardial infarctions not treated by anticoagulants. There were 4 reinfarctions during the post-infarction period in hospital, and in every one of these we found, 12 to 24 hours before the accident, a marked shortening of coagulation time. In 3 of these 4 cases we started anticoagulants at

Professor DOUGLAS — There is a big difference in the incidence of fatal post-operative pulmonary embolism between abdominal and thoracic operations. In the former it is about 2%, while in the latter in 400 consecutive thoracotomies for cardiac disease it was nil. Hence systemic causes are unlikely to be the whole answer. Perhaps mild inflammatory lesions around the large veins in the pelvis are important ætiologically.

The value, or lack of it, of anticoagulants in occlusive arterial disease of the legs is quite unknown. But it could be well settled by a double blind trial in any vascular clinic if it were well controlled by arteriographic studies.

Professor OWREN — The question was raised whether anticoagulant therapy influences angina pectoris. Waaler (Waaler, B. A. (1957), Acta Med. Scand., 157, 289) found that 40-50% of the patients were improved, a distinctly higher percentage than found with a placebo. Improvement occurred gradually during the first 12-18 months and then persisted, a finding which contradicts a placebo effect. A placebo effect was also often observed, but this effect disappeared after a few weeks. In Borchgrevink's study (Acta Med. Scand., in print) the effect on angina pectoris was analysed by the double blind technique and the results confirmed Waaler's findings. The effect may possibly be explained by the prevention of new thrombotic occlusions, thereby favouring an undisturbed development of the collateral circulation. Improvement of symptoms was much more frequent in cases with duration of symptoms of less than 2 years before therapy and in patients who kept up regular exercise, which may also promote improvement of the collateral circulation.

Professor HILL — A trial of long-term anticoagulant therapy in angina pectoris is sound if survival of the patients is made the yardstick of success. The difficulties of assessing subjective improvement (pain) are well known, for example from the critical work of Cole. Repeated testing by exercise tests, which can be very dangerous, is unjustifiable and profitless if the aim is simply to measure survival. Moreover, pain may disappear because the muscle is dead.

Dr MITCHELL — Many people feel that exercise tests in angina pectoris are dangerous. If it is thought that the test may produce infarction, it should be remembered that Master showed that there was no correlation between physical activity and the onset of an infarct, since the incidents occurred randomly throughout the day. As Wayne and Laplace pointed out, one is asking the patient to do no more, while under supervision, than he does several times a day on his own.

Professor BOYD — Is it necessarily myocardial necrosis that causes death under such circumstances?

Professor HILL — I myself have seen a patient die immediately after such a test, and have heard of others.

Dr MITCHELL — I wonder whether it is fear of the event, or of the event occurring in the laboratory, that worries the doctor.

Professor BOYD — Do domestic and other accidents in patients on long-term anticoagulant therapy constitute a serious hazard?

Dr RICHARDS — With regard to hæmorrhagic complications of therapy, minor domestic accidents often give rise to minor hæmatomata, but these are seldom serious.

I agree that the overall incidence of cardiac rupture is about 10% of myocardial infarctions coming to autopsy and that a study of all cases of rupture does not indicate any connection with anticoagulant therapy. With regard to the duration of illness to death in the cases of cardiac rupture, although this is longer than usual in the ruptures in the anticoagulant group, if the 4 cases of rupture in patients dying within 24 hours of admission are included the mean time to death comes down to 2 days.

My series does not include a high proportion of patients with multiple infarcts. The high death rate is mainly due to the many patients who died soon after admission and is similar in this respect to the series of Honey and Truelove.

[illegible]

Dr BARHAM CARTER — My first series did not constitute a strictly controlled trial, but Dr Mitchell is doubtless glad to note that my later trial was randomised.

Neurologists are more fortunate than cardiologists in being able to measure objectively disabilities short of death, and I think that the extent of a paralysis is objective enough. I have never thought that a blind trial is necessary when this type of objective measurement is possible.

Professor BOYD — I dislike the use of anticoagulants in phlebitis, superficial or deep, of the legs. I have treated phlebitis for 30 years and never [illegible] that anticoagulants might disintegrate or loosen a recent clot.

I myself do not treat peripheral arterial embolism with anticoagulants but have seen Cid Dos Santos of Lisbon's cases of peripheral embolism treated by heparin: arteriograms showed disintegration or solution of the thrombus, the patency of the arterial lumen becoming completely restored.

I have not used anticoagulants in atherosclerosis of the legs, but I have followed up 1440 patients with atherosclerosis and intermittent claudication but without gangrene or pregangrenous changes, in order to study the natural course of the disease. After 15 years, 85% of the deaths were due to atherosclerosis: 75% to "coronary thrombosis" and 10% to cerebral vascular catastrophes. Of the survivors, 10% had a non-fatal coronary or cerebral accident in the first 5 years, rising to 20% after 10 years. These complications might possibly have been prevented by anticoagulant therapy.

[illegible] vessels. Rather strict criteria in my selection of patients limit the number in whom the operation [illegible]

[illegible] a number of fatal cases, but at post-mortem death was found to be due to myocardial infarction. I am astounded by the high incidence of pulmonary embolism in some American papers. There seems to be a marked geographical and seasonal distribution of this complication.

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Besides the indications for long-term anticoagulant treatment, there is an urgent need for proper evaluation of coumarin drugs combined with vasoactive substances. New and simple methods for early detection of minor bleeding tendencies should be developed as a *safety measure*.

[illegible]

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A New Heparinoid Anticoagulant

F. K. Beller — Universitats-Frauenklinik, Tubingen

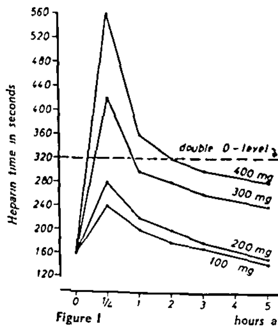
Heparinoids are synthetic or semi-synthetic compounds with biological effects like alpha-heparin Further information on the therapeutic [illegible] from [illegible] from [illegible] have [illegible] e are [illegible] ecular natural substances, the anticoagulant effect is increased, but the side-effects are also increased. To obviate this vicious circle, the compromise was attempted of increasing the anticoagulant action only to that point where the side-effects were still tolerable. Therefore, the potency of heparinoids is less than that of heparin, so that the same therapeutic effect needs more drug and toxic doses are more easily reached. Thus the therapeutic index is lower than heparin.

G 31, 150 (Geigy) is the calcium complex of the sulphated oxidative breakdown products of polygalacturonic acid methyl ester, with a calcium content of 4.5% and an average molecular weight of 40,000 It contains 5.4% methoxyl groups and has a sulphur content of 13%.

This product has excellent clinical tolerability Its anticoagulant effect was tested by thromboelastography as described by Hartert Heparin or heparinoid is mixed with platelet-rich and platelet-poor plasma, with and without incubation, and the TEG done. In citrated platelet-rich plasma a heparinoid solution of 1.1000-1 1250 is equivalent in effect to a heparin dilution of 1·4000, which corresponds to a ratio of approximately 1·3.5 in vitro. This compares very favourably with other heparinoids.

But this in vitro effect cannot be compared with the in vivo effect. The effects of single intravenous doses were measured by Howell's recalcification time (Fig. 1). Only after 0.3-0.4 g. is the initial [illegible] plastin which depends on the preparation used (Fig 2).

We adopted, therefore, a scheme of treatment (Fig. 3). The ratio of effect in the body compared [illegible] it is instead [illegible] parinoid are [illegible] ients. Most [illegible] two from recent infarction and twenty-five from so-called congestion states in abdominal carcinoma; we previously established that these swellings remitted under heparin and at least partially under heparinoids. The results where heparinoid was injected for 4-6 days and then the drug changed to Sintrom correspond to well-known experience with heparin. Mild cases do with three daily doses, the minimum in surgical patients. As described by Merz, crisis



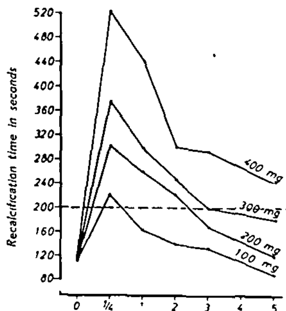


Figure I

hours after injection

PTA, Christmas Factor (IX), Proaccelerin (V), and AHG (VIII) in order of decreasing sensitivity. It also inhibited the surface activation of Hageman Factor.

In other experiments various quantities of heparin (2.5, 5, 10 and 20 units) were added to 10 ml. of human oxalated blood. After 30 minutes' incubation at room temperature, samples of heparinised blood were centrifuged and clotting factors were determined in plasma diluted 1 : 10

[illegible] factor [illegible] parent [illegible] AHG, was [illegible]

It is well known that human serum, like platelets, shows antiheparin activity. According to O'Brien (1960) this activity is related to the presence of some platelet proteins in serum (platelet-like activity of serum) and to Christmas Factor activity. O'Brien (1960) suggests that heparin neutralises Christmas Factor and platelets giving complexes with these substances.

Other findings are, however, *contradictory* Poller (1960) found that serum antiheparin activity is not related to the activities of Factor VII, IX and X in the serum.

We compared the antiheparin activities of a platelet suspension 300,000 per cu mm. with normal human serum and with Christmas serum. In the same experiment both sera were adsorbed on $BaSO_4$. The $BaSO_4$ precipitate was eluted with sodium citrate and the eluates examined The antiheparin activity of platelet suspensions and of serum was found to be of the same order of magnitude (Fig. 4). Serum antiheparin activity is not related to Christmas Factor activity, in spite of the powerful inhibitory action of heparin on this factor. Similarly there is some evidence that serum antiheparin activity is not related to Hageman Factor.

[illegible] spite of the great amount of work done on this subject.

Our findings are that heparin does not inhibit prothrombin and complex VII, but it inhibits Factor V, VIII, IX, PTA and Hageman. These factors are pa [illegible] system." Hep [illegible] inhibitor of th [illegible] On the contr [illegible] considered rat [illegible] blood-clotting [illegible] combined ther [illegible] therapy.

ANTIHEPARIN ACTIVITY OF SERUM, SERUM FRACTIONS AND PLATELETS

INCUBATION MIXTURE	HUMAN CITRATED PLASMA	THROMBIN	CLOTTING TIME
0.2 ml SALINE	0,3 ml	0,1 ml	10 "
0,1 ml SALINE 0 1 ml HEPARIN	0 3 ml	0,1 ml	60 "
0,1 ml PLATELETS 0,1 ml HEPARIN	0,3 ml	0,1 ml	12 "
0,1 ml NORMAL SERUM 0,1 ml HEPARIN	0,3 ml	0,1 ml	12½ "
0,1 ml HEMOPHILIA B SERUM 0,1 ml HEPARIN	0,3 ml	0,1 ml	10½ "
0,1 ml $BaSO_4$ ELUATE (NORMAL) 0,1 ml HEPARIN	0,3 ml	0,1 ml	17 "
0,1 ml $BaSO_4$ ELUATE (HEMOPHILIA B) 0,1 ml HEPARIN	0,3 ml	0 1 ml	15 "

Figure 4

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A New Heparinoid Anticoagulant

F. K. Beller — Universitats-Frauenklinik, Tubingen

Heparinoids are synthetic or semi-synthetic compounds with biological effects like alpha-heparin. Further information on the therapeutic action of the heparin group is derived not only from their varying effects on coagulation, but also from their side-effects. Only a few such products have attained clinical significance, and even these are disputed. By sulphation of certain high-molecular natural substances, the anticoagulant effect is increased, but the side-effects are also increased. To obviate this vicious circle, the compromise was attempted of increasing the anticoagulant action only to that point where the side-effects were still tolerable. Therefore, the potency of heparinoids is less than that of heparin, so that the same therapeutic effect needs more drug and toxic doses are more easily reached. Thus the therapeutic index is lower than heparin.

G 31, 150 (Geigy) is the calcium complex of the sulphated oxidative breakdown products of polygalacturonic acid methyl ester, with a calcium content of 4.5% and an average molecular weight of 40,000. It contains 5.4% methoxyl groups and has a sulphur content of 13%.

This product has excellent clinical tolerability. Its anticoagulant effect was tested by thromboelastography as described by Hartert. Heparin or heparinoid is mixed with platelet-rich and platelet-poor plasma, with and without incubation, and the TEG done. In citrated platelet-rich plasma a heparinoid solution of 1:1000-1:1250 is equivalent in effect to a heparin dilution of 1:4000, which corresponds to a ratio of approximately 1:3.5 in vitro. This compares very favourably with other heparinoids.

But this in vitro effect cannot be compared with the in vivo effect. The effects of single intravenous doses were measured by Howell's recalcification time (Fig. 1). Only after 0.3-0.4 g. is the initial value doubled at three hours, the usual prerequisite [illegible]

[illegible] (Fig. 2).

We adopted, therefore, a scheme of treatment (Fig. 3). The ratio of effect in the body compared [illegible] t is instead [illegible] arinoid are [illegible] ients. Most [illegible] two from recent infarction and twenty-five from so-called congestion states in abdominal carcinoma; we previously established that these swellings remitted under heparin and at least partially under heparinoids. The results where heparinoid was injected for 4-6 days and then the drug changed to Sintrom correspond to well-known experience with heparin. Mild cases do with three daily doses, the minimum in surgical patients As described by Merz, crisis

The effect of different doses on the heparin time mean value of 5 cases

The effect of different doses on the recalcification time mean value of 5 cases

Figure 1

hours after injection

PTA, Christmas Factor (IX), Proaccelerin (V), and AHG (VIII) in order of decreasing sensitivity. It also inhibited the surface activation of Hageman Factor.

In other experiments various quantities of heparin (2.5, 5, 10 and 20 units) were added to 10 ml. of human oxalated blood. After 30 minutes' incubation at room temperature, samples of heparinised blood were centrifuged and clotting factors were determined in plasma diluted 1 : 10.

Heparin added in vitro to blood inhibits clotting factors in a similar manner to heparin injected parenterally (Fig. 3). It should be noted that AHG was more inhibited in vitro than in vivo.

It is well known that human serum, like platelets, shows antiheparin activity. According to O'Brien (1960) this activity is related to the presence of some platelet proteins in serum (platelet-like activity of serum) and to Christmas Factor activity. O'Brien (1960) suggests that heparin neutralises Christmas Factor and platelets giving complexes with these substances.

Other findings are, however, contradictory. Poller (1960) found that serum antiheparin activity is not related to the activities of Factor VII, IX and X in the serum.

We compared the antiheparin activities of a platelet suspension 300,000 per cu. mm. with normal human serum and with Christmas serum. In the same experiment both sera were adsorbed on $BaSO_4$. The $BaSO_4$ precipitate was eluted with sodium citrate and the eluates examined. The antiheparin activity of platelet suspensions and of serum was found to be of the same order of magnitude (Fig. 4). Serum antiheparin activity is not related to Christmas Factor activity, in spite of the powerful inhibitory action of heparin on this factor. Similarly there is some evidence that serum antiheparin activity is not related to Hageman Factor.

spite of the great amount of work done on this subject.

therapy.

ANTIHEPARIN ACTIVITY OF SERUM, SERUM FRACTIONS AND PLATELETS

INCUBATION MIXTURE	HUMAN CITRATED PLASMA	THROMBIN	CLOTTING TIME
0.2 ml SALINE	0.3 ml	0.1 ml	10"
0.1 ml SALINE 0.1 ml HEPARIN	0.3 ml	0.1 ml	60"
0.1 ml PLATELETS 0.1 ml HEPARIN	0.3 ml	0.1 ml	12"
0.1 ml NORMAL SERUM 0.1 ml HEPARIN	0.3 ml	0.1 ml	12½"
0.1 ml HEMOPHILIA B SERUM 0.1 ml HEPARIN	0.3 ml	0.1 ml	10½"
0.1 ml BaSO₄ ELUATE (NORMAL) 0.1 ml HEPARIN	0.3 ml	0.1 ml	17"
0.1 ml BaSO₄ ELUATE (HEMOPHILIA B) 0.1 ml HEPARIN	0.3 ml	0.1 ml	15"

Figure 4

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Example : 100 mg. = 1 ml. of the 10% solution contained in the ampoules.

1st Day		2nd Day		3rd Day	
8.00h	400 mg. i.v.	8.00h	400 mg. i.v.	8.00h	400 mg. i.v.
13.00h	300 mg. i.v.	13.00h	300 mg. i.v.	13.00h	200 mg. i.v.
17.00h	200 mg. i.v.	17.00h	200 mg. i.v.	17.00h	200 mg. i.v.
21.00h	300 mg. i.v.	21.00h	300 mg. i.v.	21.00h	200 mg. i.v.

4th Day		5th Day		6th Day	
8.00h	400 mg. i.v.	8.00h	400 mg. i.v.	8.00h	400 mg. i.v.
13.00h	200 mg. i.v.	13.00h	200 mg. i.v.	13.00h	200 mg. i.v.
18.00h	300 mg. i.v.	18.00h	300 mg. i.v.		
			+ cumarin		+ cumarin

In some instances various routes of application (i.v., s.c., i.m.) were successfully used for the individual doses.

Figure 3

The effect of a combination of intravenous and subcutaneous injection of Heparinoid on the heparin time

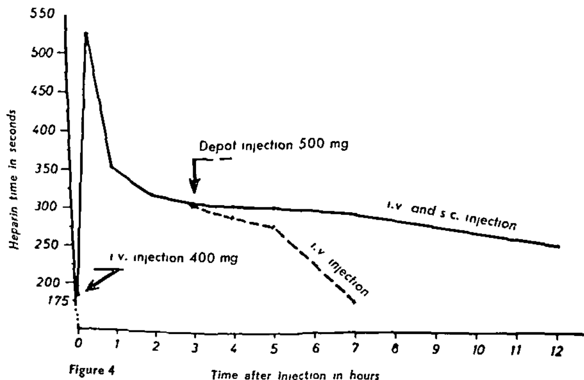


Figure 4

and lysis took place at latest six days after the beginning of therapy, when the patients were able to get up. *In six years we had only one failure: a woman* with puerperal sepsis, who died with extensive thrombosis following an injection of Pyrexal; a Schwartzman phenomenon may have occurred.

Fifteen pregnant women were treated only with heparinoid G 31 150, in slightly lower daily dosage. Pregnancy is the only condition where treatment was continued longer than six days, though with gradual reduction in dosage. All gave birth to living children; one child was deformed and died after three days. *The rest were in no way abnormal. In* three cases premature labour started during treatment. Therapy was continued to 10 minutes before parturition, when the effect was interrupted with protamine sulphate. The post partum period was normal.

One gram of a depot preparation may be injected, corresponding to the daily intravenous dose, but no absorption of therapeutic significance occurs within two hours. There was no difference in the effect of subcutaneous and intramuscular depot injections. [illegible]

The excretion is similar to that of heparin, but more biologically active substance is present in the urine than after heparin. This is clearly seen with depot heparinoid. After injection of alpha-heparin, the remaining biological activity is approximately 1/3-1/4 of the excreted amount, after heparinoid approximately one-half. That resembles results with other heparinoids, and these substances are probably *less broken down in the body* than heparin. The excretion values also confirm the findings with respect to saturation dose and thus support the dosage scheme.

In our experience, the following side-effects are frequent after the heparinoids investigated hitherto: after rapid injection: tachycardia, feeling of oppression in the chest, nausea, vomiting, cyanosis, later paræsthesiæ. We observed none of these even after *rapid intravention injection of* G 31, 150 in doses up to 1.5 g. The incidence of side-effects is so far under 1%. However, Stamm reports more frequent side-effects during longer therapy. We have no experience of long-term therapy, and it seems inadvisable to prolong treatment beyond eight days.

A special problem is loss of hair, which is particularly prominent in gynæcology and obstetrics. We observed it 8-12 weeks after the beginning of therapy even with heparin in approximately 70% of cases, and after heparinoids in almost 100%. Of these again 30-40% have total alopecia. After Heparinoid Geigy, counting distinctly visible reduction of hair, it occurred in approximately 80% and total alopecia in under 5%. In all, the hair grew again normally.

This product may be used where there is heparin allergy, but it will find its chief place where heparin is prohibited or limited *by its price. It is of great value* for the initiation of thrombosis treatment, and is worth including in our therapeutic equipment.

Prothrombintime (Quick) in dependence on the thromboplastin.

1, 2, 3 and 4 different thromboplastinpreparations

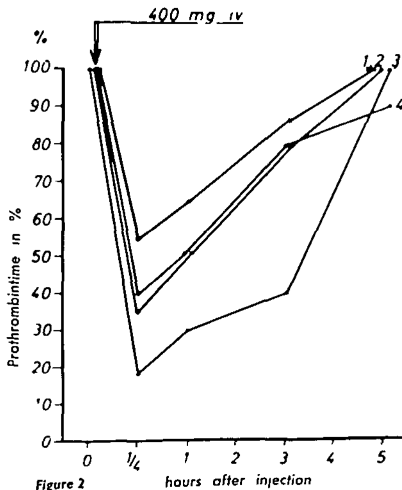
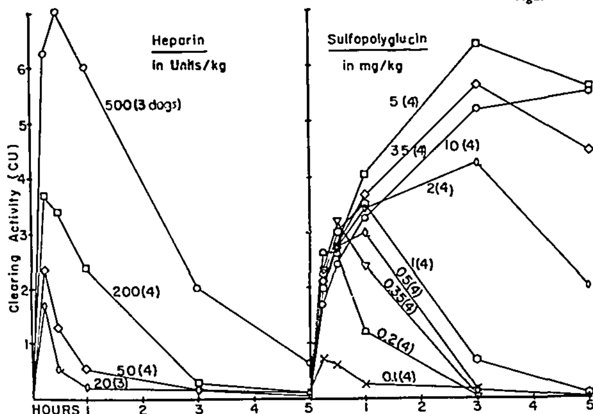


Figure 2



"Ediol," a stabilised 50% coconut oil emulsion, when tested
activities we
the use of
(obtained t
Institute for

Free fatty acids, before and after incubation of plasma with Ediol or human low-density Beta-

Mongrel dogs were fasted overnight (about 20 hours) with access to water The drug was con-

Intravenous SPG v Heparin

Heparin was injected i v into dogs in groups of 3 or 4 at doses of 500 to 20 USP units/kg, all doses being given concurrently. SPG was similarly injected i v. into groups of 4 dogs at doses of 10 to 0 1 mg./kg. In all cases blood samples were taken before, and ¼, ½, 1, 3 and 5 hours after injection for determination of LCA and CT.

With respect to anticoagulant effect (Fig. 1), the time effect curves for heparin and SPG were qualitatively similar, both series showing rapid peaks, and slower disappearance of effect. Heparin had a much greater anticoagulant effect than SPG. On a weight basis, it was approximately five to seven times more active than SPG. This agrees very well with the figure of 21 USP units per mg, which was found for SPG in vitro

LCA time-effect curves (Fig 2) resulting from i v injection of heparin and SPG differed both quantitatively and qualitatively. Like the anti-

In a study in which radioactive SPG containing

A New Orally Effective Heparinoid

ANTICOAGULANT AND ANTILIPÆMIC ACTIVITY IN ANIMALS AND MAN AFTER ORAL AND PARENTERAL ADMINISTRATION

E. WINDSOR and G. E. CRONHEIM—Research Division, Riker Laboratories, Inc., Northridge, California

The present report describes the results with a new heparinoid which is absorbed following oral dosage. The drug has been successfully *administered* either parenterally or orally or both, to rats, rabbits, cats, dogs, monkeys, and to man.

Experiments designed to learn the effects of prolonged administration of heparinoids on lipid metabolism have been hampered *in the past by*

The present report is limited to ...

Materials and M...

Riker 560 is a mixture by weight of 4 parts of SPG potassium and 1 part of disodium dihydrogen ethylenediamine-tetraacetate (" Versenate " sodium).

Heparin Sodium USP for parenteral administration was " Lipo-Hepin " (Darwin), 10,000 USP units per ml., diluted with physiological saline to a final volume of 0.5 ml/kg. Heparin sodium USP was also used as a powder, with a potency of 130 USP units per mg.

Whole blood ...

mean CT.

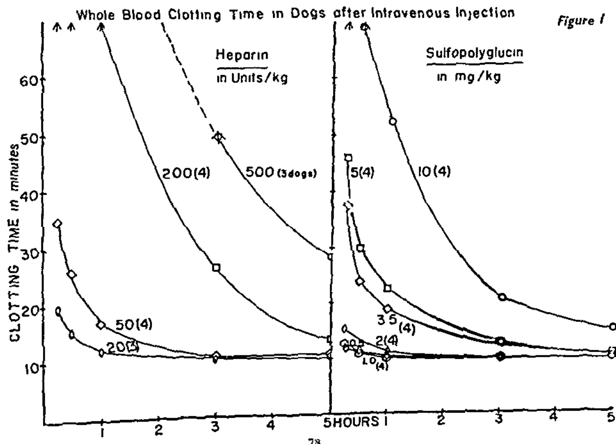
L...

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where

in optical density of enzyme-substrate mixture *incubated at 20°C,* and read at 700 mu) The only modifications were the limitation of reaction time to 10 minutes, and the use of " Ediol " (SchenLab), diluted 1 to 50 with water, as a substrate. With



below 1 mg./kg. Comparing the lipolytic effects of each drug by the two modes of injection, subcutaneous SPG at 1 mg./kg or more was more effective than i.v. SPG, while s.c. heparin showed less lipolysis than i.v. heparin at all doses.

Oral Riker 560, SPG, and Heparin.

SPG and Riker 560 were given orally to fasted dogs at doses of 100 to 1000 mg./kg. and 25 to 200 mg./kg. respectively. Anticoagulant effects (Fig 6) appeared with considerable delay compared to those after i.v. administration, while LCA *time-effect curves* were qualitatively similar to results after i.v. injection. SPG doses below 300 mg./kg., and Riker 560 doses of 100 mg./kg. or less had little or no effect on CT. However, all doses produced considerable increases in LCA. LCA and CT dose-response curves at 3 hours *after the drugs (Fig 7)* were parallel, and indicated that the addition of Versenate sodium to SPG increased the absorption from the gut about five-fold. Comparing the lipolytic effects of oral Riker 560 with parenteral SPG or Riker 560 one derives the value for gastrointestinal absorption of *two to three per cent. of* the oral dose.

After the increased absorption of SPG by the addition of Versenate sodium had been established, it was found that the same adjuvant would bring about the absorption of heparin from the gastro-intestinal tract, as shown by *CT and LCA* measurements.

Both LCA and CT effects were delayed, as compared with

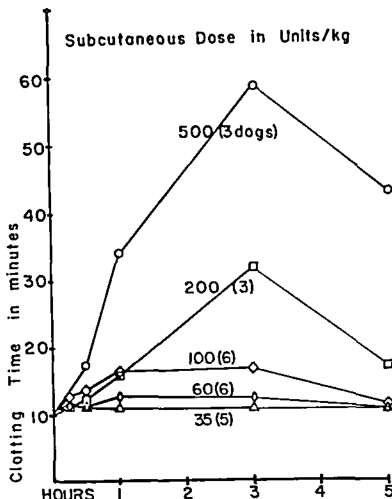


Figure 4

per cent of the administered dose.

No changes beyond control levels were seen in Quick prothrombin time, or "Thrombotest" time after peroral heparin, heparin + Versenate, Riker 560, or Versenate.

SPG alone required a minimum of 20 g. to *produce an antilipæmic effect.* The addition of Versenate (potassium in Series A, sodium in Series B) increased absorption of SPG approximately five-fold at a ratio of SPG : Versene of 4·1. This ratio was selected because higher ratios reduced the utilisation of SPG and lower ratios limited the total dose of SPG that could be administered due to side effects of Versene.

Exploratory studies have indicated that Riker *560 at a dose of 4 + 1 g. induced* LCA levels of the same order of magnitude as that seen in man after subcutaneous doses of heparin of 10,000 USP units, but without the concomitant anticoagulant effects. The LCA responses were dose-related and the peak occurred more rapidly than had been expected after experiments with dogs.

Fasting conditions promoted the best absorption of the drug, a slight decrease in LCA being seen if *given with a carbohydrate meal,* and a considerable

S^{35} was injected into dogs (Fig. 3) radioactivity, metachromasia, and prolonged anticoagulant effect all disappeared from the blood at the same rate (Levy et al. 1960), indicating that the presence of SPG was required to produce the anticoagulant effect. This had previously been shown to be true for heparin by Fiber and
the highe
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Subcutaneous SPG v. Heparin.

Heparin was injected subcutaneously at doses of 500 to 35 USP units/kg. into groups of 3 to 6 dogs. Similarly, SPG was injected at doses of 5 to 0.35 mg./kg. Blood was sampled before and ¼, ½, 1, 3 and 5 hours after injection for LCA and CT determination

No prolongation of CT after subcutaneous SPG was seen, even at the highest dose of 5 mg./kg. At the same weight of heparin (500 U/kg.) (Fig. 4), there was a fairly rapid rise in CT to more than

500 U/kg. LCA at 5 hours was still 50% of the maximum effect.

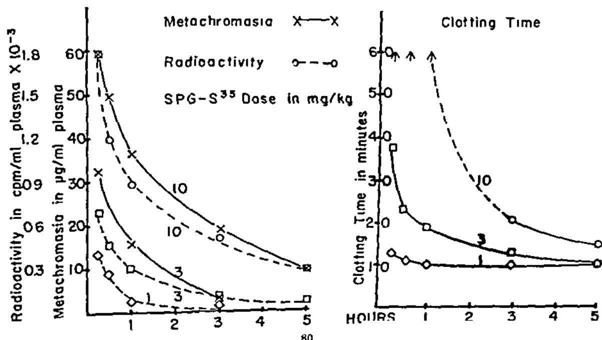
After s.c. SPG at low doses (up to 1 mg./kg.) maximum LCA was also seen within ½ to 1 hour. However, levels were about 3 times higher than after the same heparin doses and fell gradually. At 5 hours after 1 mg./kg. the LCA level was still

Characterization of Lipolytic Activity.

In order to determine substrate specificity of the lipæmia-clearing enzyme system in plasma, post-SPG dog plasmas were incubated at 37° with either human low-density Beta-lipoproteins or with Ediol. Free fatty acid (FFA) was titrated before and after 1 hour of incubation at 37°C and the difference was calculated as microequivalents per litre per hour. For the same 19 plasma samples the levels of CU were also determined

A correlation was found to exist between the two measurements, 1 CU being equal to 1.3 +/— 0.1 uEq/L/hr., with the Beta-lipoprotein substrate No difference was seen in CU measurements between Beta

Intravenous Radioactive Sulfopolyglucin in Dogs *Figure 3*



below 1 mg./kg. Comparing the lipolytic effects of each drug by the two modes of injection, subcutaneous SPG at 1 mg./kg. or more was more effective than i.v. SPG, while s.c. heparin showed less lipolysis than i.v. heparin at all doses.

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Clotting Time in Dogs after Heparin

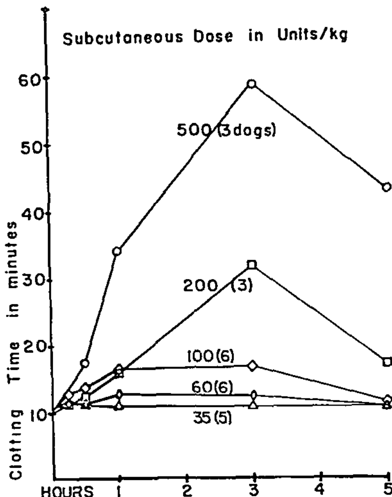


Figure 4

SPG alone required a minimum of 20 g. to produce an antilipaemic effect. The addition of Versenate (potassium in Series A, sodium in Series B) increased absorption of SPG approximately five-fold at a ratio of SPG Versene of 4:1. This ratio was selected because higher ratios reduced the utilisation of SPG and lower ratios limited the total dose of SPG that could be administered due to side effects of Versene.

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No changes beyond control levels were seen in Quick prothrombin time, or "Thrombotest" time after peroral heparin, heparin + Versenate, Riker 560, or Versenate.

Human Pharmacology.

Riker 560 and SPG were administered orally to 76 healthy volunteers in 132 single doses ranging from 1 to 20 g. of SPG, in 2 series of trials (Table 1). Only a single case was observed in which whole blood clotting time was prolonged. It reached 1.5 times the control level 2 hours after a dose of 4 g. of SPG plus 2 g. of Versenate potassium, and it returned to the control level at 4 hours.

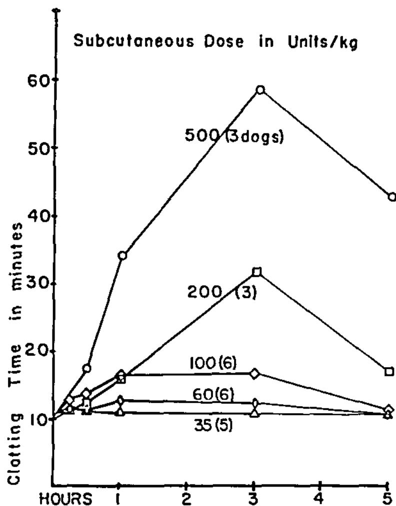


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Subcutaneous SPG v. Heparin.

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No prolongation of CT after subcutaneous SPG was seen, even at the highest dose of 5 mg./kg. At the same weight of heparin (500 U/kg.) (Fig. 4), there was a fairly rapid rise in CT to more than 3 times

LCA was still slightly elevated at 5 hours and at 500 U/kg. LCA at 5 hours was still 50% of the maximum effect.

After s.c. SPG at low doses (up to 1 mg./kg.) maximum LCA was also seen within ½ to 1 hour. However, levels were about 3 times higher than after the same heparin doses and fell gradually. At 5 hours after 1 mg /kg. the LCA level was still greater than 1 CU. At higher does of 2 and 5 mg /kg. the LCA levels continued to rise for 5 hours The course after that time is unknown, but at 24 hours after injections LCA had returned to control values.

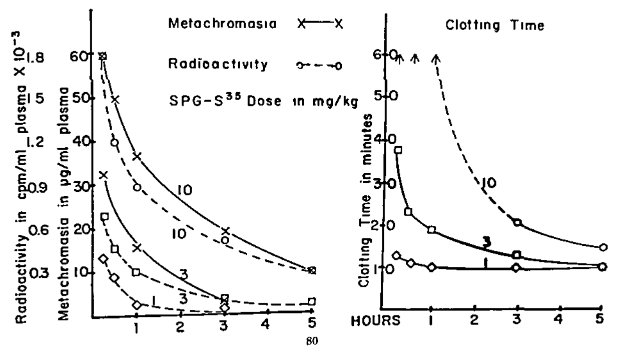
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A correlation was found to exist between the two measurements, 1 CU being equal to 1 3 +/— 0 1 uEq/L/hr., with the Beta-lipoprotein substrate No difference was seen in CU measurements between Beta-lipoprotein or Ediol substrates.

Becau the LCA and hepar with one

sampling period, it was assumed, for purposes of computation, that the rate of LCA decrease was the same as those observed at lower doses



Lipemia Clearing Activity in Dogs after Subcutaneous Injection

Heparin in Units/kg

Sulfopolyglucin in mg/kg

Clearing Activity (CU)

500(3)
200(3)
100(6)
60(6)
35(5)

5 (2 dogs)
2(4)
1 (3)
0.6 (3)
0.35(2)

HOURS 1 2 3 4 5 1 2 3 4 5

Figure 5

Figure 6

● ○ EDTA
■ □ No EDTA
—— Clearing
– – Clotting

Clearing Units

Minutes

mg / kg

lowering if given with a fatty meal. Delaying the fatty meal for 45 minutes did not improve absorption of the drug.

Plasma samples drawn during series A (Table 2) were incubated with an Ediol substrate and the increase in release of free fatty acid was measured. While 4 g. of SPG alone had no effect on lipolysis, the same dose when given with Versenate increased free fatty acid by approximately eight-fold at 2 hours after the drug.

Trials of Riker 560 at daily dose levels of 2 g. are now in progress. In this and other trials to be reported later it has been found that at dose levels too low to show much change in plasma LCA, significant lowering of serum low-density Beta-lipoproteins occurs

Discussion

Most of the measured differences between SPG and heparin are quantitative rather than qualitative. Thus their influence on coagulation of blood seems to be dependent on the presence of the drug, as

greater degree than heparin and consequently is a much stronger anion. Nevertheless, its potency is only about one-fifth that of heparin

Even the slight anticoagulant effect seen after i.v. injection can be further reduced by slowing the rate at which SPG appears in the bloodstream, as by subcutaneous injection, or by absorption through the gut.

The rates of appearance in the plasma of the

amount to produce the enzyme which when exhausted reappears at a limiting rate, or, SPG replaces heparin which then extracts the enzyme from depots such as fat tissue. Clearly, there is a need for further research on the mechanisms in-

heparin

magnesium in the intestinal tract since the ... lar chelates of Versene were inactive, while the alkaline salts were active.

The ability to bring about the appearance

TABLE 1

LIPÆMIA CLEARING ACTIVITY IN MAN AFTER SULPHOPOLYGLUCIN PLUS VERSENE

Dose in g. SPG	Dose in g. Versene	Experimental Condition	No of Persons	Lipæmia Clearing Activity x 100 (CU.) (mean and range) 0 Hr.	2 Hrs.	4 Hrs.
SERIES A: SPG, +/— Potassium Versenate.						
20	—	Fasting	4		84 (41-133)	
4	—	Fasting	3		4 (0-8)	
4	2	Fasting	5	1 (0-5)	184 (139-267)	74 (10-268)
4	1.33	Fasting	4	1 (0-4)	97 (5-304)	30 (2-108)
10	0.5	Fasting	4		22 (7-52)	
SERIES B: SPG, +/— Sodium Versenate.						
10	—	Fasting	4	0	11 (0-22)	13 (0-28)
5	—	Fasting	4	2 (0-4)	3 (0-11)	10 (0-17)
4	1	Fasting	12	6 (0-50)	76 (0-150)	55 (0-175)
2	1	Fasting	8	4 (0-17)	71 (4-265)	42 (0-217)
1	1	Fasting	8	1 (0-4)	17 (0-72)	0 (0-10)
2	½	Fasting	8	2 (0-17)	33 (0-100)	8 (0-17)
1	½	Fasting	4	10 (4-22)	14 (0-50)	7 (0-17)
4	1	Carbohydrate Meal	4	0 (0-0)	58 (22-118)	14 (0-57)
4	1	Fat Meal	4	0 (0-0)	20 (0-78)	6 (0-22)
4	1	Fat Meal 45 min later.	8	10 (0-38)	29 (0-83)	9 (0-17)

TABLE 2

INCREASE IN FREE FATTY ACID DURING PLASMA INCUBATION (SERIES A).

Dose (g.) SPG	Dose (g.) Versene	No of Persons	Free Fatty Acid Release in microEq/L./hr. (mean and range) Sample taken at 0 Hours	2 Hours	4 Hours
4	0	7	185 (9-391)	240 (65-410)	
4	2	5		1820 (1530-2170)	890 (320-1450)
4	1.33	4		1910 (490-4190)	690 (270-1100)

Lipemia Clearing Activity in Dogs after Subcutaneous Injection

Figure 5

Figure 6

lowering if given with a fatty meal. Delaying the fatty meal for 45 minutes did not improve absorption of the drug.

Plasma samples drawn during series A (Table 2) were incubated with an Ediol substrate and the increase in release of free fatty acid was measured. While 4 g. of SPG alone had no effect on lipolysis, the same dose when given with Versenate increased free fatty acid by approximately eight-fold at 2 hours after the drug.

Trials of Riker 560 at daily dose levels of 2 g. are now in progress. In this and other trials to be reported later it has been found that at dose levels too low to show much change in plasma LCA, significant lowering of serum low-density Beta-lipoproteins occurs.

Discussion.

Most of the measured differences between SPG and heparin [illegible]

[illegible] greater degree than heparin and consequently is a much stronger anion. Nevertheless, its potency is only about one-fifth that of heparin.

Even the [illegible] the gut.

The rates of appearance in the plasma of the lipæmia-clearing enzyme system seem to differ following i.v injection of the two drugs. Where heparin injection is followed immediately by the appearance of LCA, SPG injection is followed by LCA expressed in delayed curves, the greater the dose, the later the peak. Some of the possible interpretations of this phenomenon are SPG may activate an inactive precursor in the tissues to form the clearing enzyme, SPG may act as a cofactor, SPG may require the presence of an unknown substance normally present in the blood in limited amount to produce the enzyme which when exhausted reappears at a limiting rate, or, SPG replaces heparin which then extracts the enzyme from depots such as fat tissue. Clearly, there is a need for further research on the mechanisms involved. However, the rate of drug appearance in the bloodstream is involved This is substantiated to some extent by the similarity in shapes of LCA curves after [illegible]

The increase in oral absorption of SPG by the addition of Versenate sodium is a new phenomenon That it is not restricted to SPG was confirmed by successful oral absorption of heparin, dextran sulphate, and polyethylene sulphonate (Windsor [illegible] alkaline salts were active

The ability to bring about the appearance

TABLE 1

LIPÆMIA CLEARING ACTIVITY IN MAN AFTER SULPHOPOLYGLUCIN PLUS VERSENE.

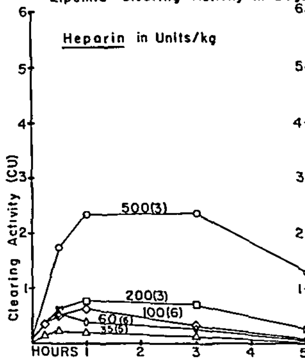
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1	1	Fasting	8	1 (0-4)	17 (0-72)	0 (0-10)
2	½	Fasting	8	2 (0-17)	33 (0-100)	8 (0-17)
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Lipemia Clearing Activity in Dogs after Subcutaneous Injection



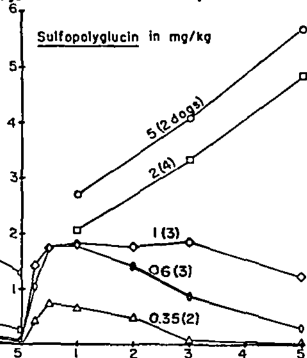


Figure 5

Figure 6

● ○ EDTA
■ □ No EDTA
—— Clearing
– – Clotting

Clearing Units: 1 2 3 4 5

Minutes: 10 20 30 40 50

mg / kg: 25 50 100 200 500 1000

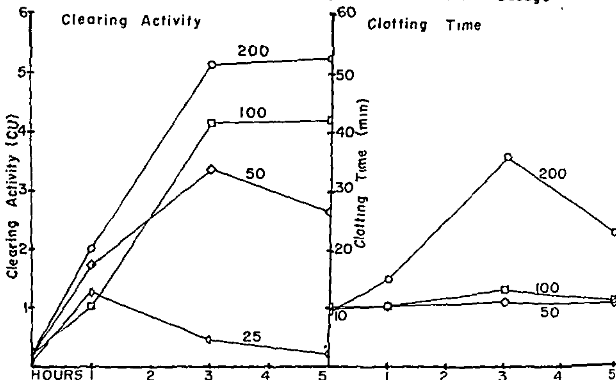


Figure 7

in the blood of a lipæmia-clearing lipase by an oral drug without the necessity for concern about increasing coagulation times should stimulate further research into various disorders of human lipid metabolism.

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DISCUSSION

Sir JAMES LEARMONTH—I shall confine my remarks to the place of anticoagulants in peripheral occlusive arterial disease, in which I have most experience.

First, I wish to point out that, in addition to the phenomenon of thrombosis occurring in a patient

With regard to the use of anticoagulants, of

value, and the complexity of these factors seems to

here is the same as that determined in a specimen of systemic blood However, in any collateral system

origin This may be because the structure of the walls

of

ver

insertion of a large

Dr RILEY — Looking at the mast cell story in retrospect

of anticoagulant. With the discovery of heparin

ponse to the presence of a clot—more properly perhaps a thrombus.

appreciated that the heparins of some species have

clotting.

This leads me to my only question. I wonder whether an attempt has been made to suppress the ionization of heparin by esterifying the remaining half-esters of sulphuric acid with organic groups which would also render the molecule more fat-soluble? Such a heparin, if absorbed, would no doubt be trapped in the fat depots. There, with the aid of a friendly esterase, the original sulphated

mast cells that normally dwell in the connective

offer a simple explanation for the only situation in which heparin is known to enter the blood in quantity, namely the blood of the dog in peptone or anaphylactic shock

organ.

It is because of Dr Windsor's

I would

other ar

instead (

merely a

this species.

Dr VERSTRAETE—A third alternative besides surgery and anticoagulants can be considered in the

treatment of recent peripheral vascular occlusion: the administration of lytic agents. Aortograms performed on a 72-year-old man showed acute complete occlusion of one common iliac artery and the contalateral internal iliac artery. He was given 6,000,000 units of streptokinase locally through a percutaneous catheter and subsequently 16,000,000 units intravenously, by continuous drip. He recovered gradually and the second aortogram showed that both closed vessels had become patent. All arterial pulses were felt and the patient walked home unaided.

Dr WALKER — Dr D. M. Shepherd and I made a comparative study of the lipæmia-clearing effects produced by Riker 560 (sulphopolyglucin plus EDTA adjuvant) administered orally, and sulphopolyglucin administered intravenously. Lipæmia-clearing activity was measured by the spectro-photometric method of Grossman.

A group of three rats, starved for 24 hours before use, each received by stomach tube R560 [illegible]

treated rats nor the controls showed any trace of clearing activity in the blood

A group of three rats each received by intravenous injection sulphopolyglucin 5 mg per kg. [illegible]

A similar comparative experiment was carried out in human subjects (Fig. 2) No clearing activity was produced at three hours by orally administered R560 (2 gm. sulphopolyglucin plus adjuvant), although strong anticoagulant activity at 15 minutes,

Effect of route of administration on lipæmia-clearing activity of sulphopolyglucin in rats.

	Intravenous Route		Oral Route	
Optical Densities (O D.).	SPG 5 mg /kg.	Saline Control	SPG 200 mg /kg. + adjuvant	Saline Control
Initial O.D.	0 521	0.554	0 590	0.484
O.D. after 10 min.	0.367	0.561	0 586	0 489
Fall in O D.	0 154	——	——	——

Figure 1

and good clearing activity at two hours, were obtained when sulphopolyglucin 3 mg. per kg was given intravenously.

In addition, four humans were given orally the same dose of 2 gm. sulphopolyglucin plus adjuvant, and blood was taken for examination at two and four hours; while three others received orally 1 gm. plus adjuvant six-hourly to a total of 8 gm., the blood being examined 24 hours after starting treatment and

[illegible] hours after the last dose. In only one case [illegible]

examined electrophoretically for the presence of sulphopolyglucin. No drug could be detected in these samples. On dialysis and 10-fold concentration of the dialysed urines, however, a very faint spot corresponding to sulphopolyglucin was detected.

Effect of route of administration on lipæmia-clearing activity of sulphopolyglucin in man.

	Intravenous Route		Oral Route	
Optical Densities (O.D.)	Saline Control	SPG 200 mg.	Saline Control	SPG 2 gm + adjuvant
Initial O.D.	0.691	0.508	0.637	0.573
O.D after 10 min	0.695	0 436	0 637	0.576
O D. after 30 min.	0 713	0.313	0 645	0 582
O.D. fall in 10 min.	——	0.072	——	——
O.D fall in 30 min.	——	0.195	——	——

Figure 2

Assuming a daily urinary output of 2 litres, we calculate that this would correspond to a daily urinary excretion of about 5 milligrammes of sulphopolyglucin.

These results indicate that R560 is very poorly absorbed from the gut under our conditions of [illegible]

suggests that the production of venous thrombosis [illegible]

There is no evidence that heparin has any physiological role except perhaps in fat transport [illegible]

lant.

Mr ROTHNIE — I should like to raise two points which have a bearing on the use of extracorporeal circulations. It is well known from the work

of Roskam and Hugues that heparin prevents the adhesion of platelets in vivo. However, in the heparinised blood used in the heart-lung machines [illegible] preservative in the commercial preparation? Should we therefore use freshly-made solutions of heparin?

Secondly, I should like to ask Dr Beller whether the alopecia following heparin administration is due to prolonged administration and is therefore dependent on duration of treatment or is dependent on the total dose, whatever the time factor. We had a recent patient who underwent perfusion for about one hour, followed by complete heparin neutralisation, and who developed a patch of alopecia six weeks after operation. Could this be due to heparin despite the short exposure to the drug?

Dr BELLER — On the question of whether it is better to use coumarins alone or heparin and [illegible] preparations.

With regard to hairfall, we believe that it is a problem, not of the duration of treatment, but of the dose. When a certain dose is reached, of the magnitude of which we have as yet no knowledge, hairfall occurs after eight to ten weeks. So far its origin is not clear.

Dr SHARP — I should like to ask Dr Windsor whether he has any evidence that EDTA given intravenously with heparin potentiates the action of the latter.

Dr WINDSOR — EDTA injected intravenously simultaneously with either oral or intravenous sulphopolyglucin does not potentiate antilipæmic or anticoagulant effects.

Work is being done at present by our chemists to find suitable compounds which will be less polar and therefore more easily absorbed by mouth.

If the effects I described were merely due to the histamine-binding so prevalent in dogs, thereby releasing heparin from the mast cells, in addition to the antilipæmic effects there would be concomitant anticoagulant effects in the same ratio as is observed following heparin.

The rat is an unfortunate animal for oral heparinoids since the intestinal tract is always full of fibres and food which bind the strongly charged sulphates. However, a liquid diet for 72 hours enables the rat to demonstrate its ability to absorb sulphopolyglucin.

Side-effects such as intestinal cramping are associated with large doses of Riker 560. We now find antilipæmic effects with smaller doses by measuring the decline in low-density beta-lipoproteins ultracentrifugally.

The Haematology Of Extracorporeal Circulation With And Without Hypothermia

A. A. SHARP, B. EXCELL, E. SALZMAN and O. THORUP—
Department of Hæmatology, The Radcliffe Infirmary, Oxford

The artificial extracorporeal circulation is now established as a mechanical substitute for the heart and lungs or the kidney. While such circulations have been used for many years in the treatment of uræmia, it is only in recent years that more complicated machines have been used to further the scope of cardiac surgery. There is now an established literature on this subject, but the ...

hazard is the severe hæmorrhage from the damaged tissues in the immediate post-perfusion period. Many reasons have been advanced to explain this hæmorrhagic tendency, namely, thrombocytopœnia, ...

MATERIALS

Heparin (Boots Crystalline—Cresol-free).
Hexadimethrine Bromide (Polybrene—Abbott.)
Edglugate—

EDTA (Ethylene diamine tetra acetic acid)

...

pH after autoclaving 6.7

METHODS.

Platelet Count—Brecher and Cronkite (1950)
Packed Cell Volume—Hawkesley micro-method
Thrombin Clotting Time.

0.1 ml. human thrombin (Fibrindex-Ortho) 20 units/ml. was added to 0.5 ml. plasma and the clotting time recorded.

Estimation of Fibrinogen—Thrombin Titre Method—

Sharp, Biggs, Howie and Methuen, 1958.

Plasma Fibrinolysis—Biggs and Macfarlane, 1957.
Plasma Hæmoglobin—Dacie, 1956.
Prothrombin—One-stage } Biggs and Macfarlane,
" Two-stage } 1957.
Protamine or Polybrene Titration of Plasma Heparin Levels (modification of the method of Jaques, 1943).

Serial dilutions of stock solutions of protamine ...

equivalent.

Blood heparin equivalent = plasma heparin equivalent x $\frac{100 - \text{Hæmatocrit}}{100}$

Antihæmophilic Globulin Assay—Biggs and Macfarlane, 1957.

In Vitro Hæmolysis—Modification of the method of *Stewart and Surridge, 1959.*

...

removed and the supernatant plasma examined for the amount of free hæmoglobin.

Anticoagulant For Perfusion Blood.

The extracorporeal pump used in the present series needs 8-12 pints of blood to prime before connecting to the patient's circulation Originally, fresh heparinised blood (20 mgms heparin/500 mls. blood) collected on the morning of operation was used Stored heparinised blood (30 mgms. heparin/500 ml. blood) collected the day before operation was found to be unsatisfactory as the platelet count ...

morning of the operation, the anticoagulant EDGLUGATE was substituted for heparin. Blood collected into this anticoagulant on the day before operation is heparinised and recalcified before the pump is primed. This was found to be a suitable substitute for fresh blood and does confer the additional advantage of leaving a reasonable period for adequate cross-matching of the blood 30 mgms. heparin and 5 ml 10% calcium gluconate are added to each pint just before the pump is primed.

RESULTS.

Pre-Operative.

All patients were screened for defects of their hæmostatic and coagulation mechanisms. In only 3 patients were any significant defects determined

(Table 1). Two patients had a long bleeding time. In one the defect was inherited as the mother also had a long bleeding time. These patients were given steroid therapy for one [illegible] bleeding time post-operatively, but surgery had not improved his defect. The other did not have a bleeding time performed post-operatively.

In one patient thrombocytopœnia was found, but the bleeding time was normal. No pre-operative treatment was given, and mild post-operative bleeding occurred. This patient, who was very cyanosed, also had marked secondary polycythæmia (hæmatocrit 68%). No difficulty was met in the initial stages of perfusion in spite of the obvious increased viscosity of the blood

During Perfusion.

Platelets—In all patients the platelet count dropped the moment the pump and patient's blood were mixed (Fig. 2). The degree of induced thrombocytopœnia varied, but the platelet count was always below 150,000/cu.mm. and usually below 100,000/cu mm. This thrombocytopœnia persisted all through perfusion, and was accentuated if the blood was cooled to

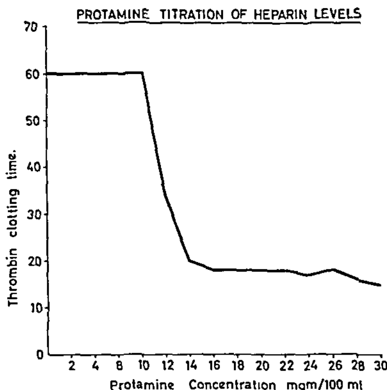


Figure 1—*Protamine and Polybrene Titration (Thrombin)* The clotting times of protamine-plasma-thrombin mixtures showing by the sudden shortening of the time ("break point") the amount of antidote equivalent to the plasma heparin.

TABLE I

Patient	Bleeding Time	Platelet Count	Diagnosis	Cyanosis	Family History	Post op Bleeding Time	Post op Haemorrhage
37 ♂	+	Normal	Mitral Stenosis	++	Nil	+	Nil
4 ♂	+	Normal	V.S.D.	-	+	?	Nil
21 ♀	Normal	24,000/ cu.mm	Fallot	++	Nil	Normal	+ -

Defects of hæmostatic mechanism discovered pre-operatively.

TABLE II

Case	Sex Age	Haemolysis	Fibrinolysis	Thrombin Titre	Defibrination	Fibrinogen Therapy	Post-Op Bleeding
5 ♂	60	71 mgm %	+++++	1/32	Nil	Nil	Nil
7 ♀	3	Nil	+++	1/16	++	2.5 Gms	Nil
10 ♂	37	++	++++	1/16	++	Nil	Nil
21 ♂	5	Nil	++++	1/64	Nil	Nil	Nil
26 ♀	17	Nil	+++++	1/16	++	5 Gms	Nil
28 ♀	21	Nil	++++	1/32	Nil	Nil	±
33 ♂	22	+	++++	1/64	Nil	Nil	Nil

The inter-relationship of hæmolysis, fibrinolysis and defibrination in 7 cases.

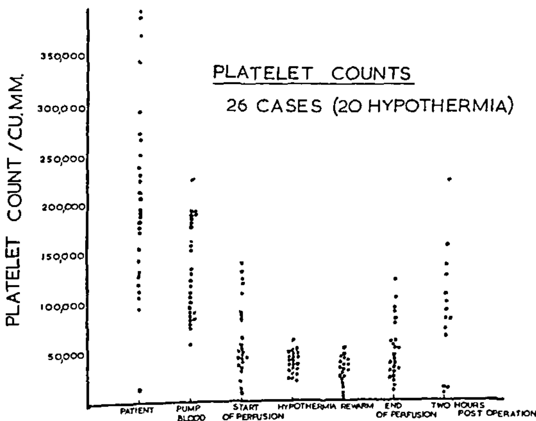


Figure 2—*Platelet counts during the sequence of perfusion in 26 cases*

[illegible]
was used.

Platelet Transfusions

As thrombocytopœnia was invariable during perfusion, platelet concentrates were prepared and [illegible]

Fibrinolysis.

In all but 4 cases some degree of fibrinolytic activity could be demonstrated. Although a relatively insensitive technique was used, the activity was very marked in 7 cases (Table II).

In 9 patients demonstrable activity was present in the patient's blood taken just before perfusion started The remainder all showed active fibrinolysis by the end of perfusion.

No one factor was discovered to accentuate fibrinolysis, although in one case the sudden need to return sucker blood to the pump was accompanied by a dramatic increase of activity. Fibrinolysis disappeared immediately after the end of perfusion.

Fibrinogen Depletion.

In 3 of the 7 cases with severe fibrinolysis evidence of fibrinogen depletion was detected (Table II). In all the defect was only partial, but in two patients concentrated fibrinogen was given prophylactically. In none of these was there any excessive post-operative hæmorrhage.

Hæmolysis.

[illegible]
hæmolysis.

"In vitro" experiments performed by the method of Stewart et al (1959) confirmed that the amount of hæmolysis is influenced by the type of plastic tubing used Stewart's findings that Tygon tubing was the best were confirmed, but British Beverage tubing was found to produce more hæmolysis than Stewart et al. (1959) measured. Autoclaving and washing after use increased the amount of hæmolysis produced by Tygon tubing

Heparin Levels.

The dose of heparin given to the patient was 3 mgms/kg of body weight. As the blood used for perfusion contains 6 mgms/100 ml, the resultant heparin level of the mixed pump-patient blood was between 4-6 mgms, depending on the blood volume of the patient Therefore, the amount of heparin in the blood was estimated by protamine or Polybrene titation at intervals during perfusion to determine the level of heparin at the beginning and the end of perfusion.

Protamine or Polybrene Neutralisation of Heparin.

Protamine or Polybrene was given at the end of perfusion in a dose equivalent to the total [illegible]

In some instances, this calculated dose was inadequate, and titration of heparin was found to be too insensitive for detecting residual heparin in the blood after the appropriate antidote was given. Such residual levels are usually less than 0 5 mgm. heparin/100 ml. blood.

The thrombin clotting time of plasma was found to be a sensitive indicator of such traces of heparin (Peden and McFarland, 1959). If the [illegible]

Post-Perfusion Hæmorrhage.

In the first few cases of this series, protamine sulphate was used to neutralise heparin in a dose of 1 mgm. for each mgm. of heparin equivalent that was calculated to be present in the patient at the end of perfusion Such doses were found to be inadequate and severe post-perfusion capillary bleeding occurred.

If the heparin antidote was given in amounts [illegible]

Other Coagulation Factors.

The levels of other coagulation factors were not studied after perfusion as the results of such tests must be prejudiced by the amounts of heparin or heparin antidote in the plasma. Where attempts were made to assay antihæmophilic globulin and prothrombin in patients in the post-perfusion period, only a partial depletion of these factors was found. The Quick prothrombin time was usually prolonged, as is Owren's prothrombin time, but the level of "prothrombin" has never been below 50%. In [illegible]

Serotonin and ATP.

Sarajas, Kristoffersson and Frick (1959) have [illegible]

of both these substances were performed by Dr. Blashko and Dr. G. V. R. Born on blood samples obtained in [illegible]

significant amounts of these toxic substances were detected in the plasma.

Post-Operative Period.

The rise in platelet count that develops in the immediate post-perfusion period was not sustained post-operatively. [illegible]

cytopœnia was not accompanied by a prolonged bleeding time.

Heparin was not used post-operatively, but [illegible] falling to levels below 8% (Owren and Aas, 1951). These low levels in [illegible]

secondary hæmorrhage [illegible]

Discussion

The greatest danger to the patient perfused with the [illegible]

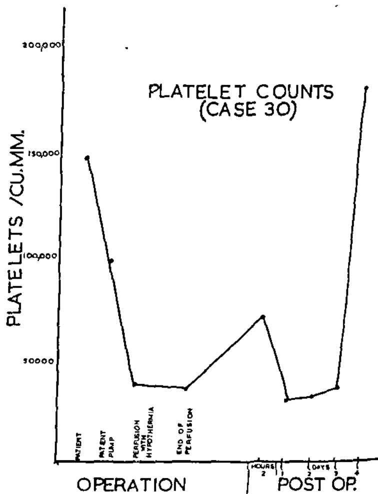


Figure 3—*Platelet counts during perfusion in the post-operative period (case 30).*

hæmorrhage from cut surfaces. This bleeding takes the form of a diffuse ooze from all cut surfaces rather than from only one bleeding point. The amount of blood lost can be a matter of pints within a few minutes. It is surprising that such diffuse hæmorrhage is not the invariable result of perfusion as the composite defect induced by thrombocytopœnia, active fibrinolysis, partial defibrination, and probable partial depletion of other coagulation factors, such as prothrombin and anti-hæmophilic globulin would, in most hæmatologists' opinion, be an adequate explanation. Yet these defects can exist together without hæmorrhage occurring.

The factor responsible for this post-perfusion [illegible]

if the antidote was given in amounts equivalent to the heparin that could be measured in the blood at the end of perfusion, it was obviously insufficient to produce adequate neutralisation of heparin. This would suggest that the fall of blood heparin was not due to destruction but to its escaping from the circulation into the extravascular tissues, whence it returns when the normal circulation is restored. Normally this reappearance of heparin in the blood is rapid, but, if delayed, it could be an explanation of the "rebound phenomenon."

Heparin is an inefficient anticoagulant for preservation of stored blood, but it is the only one available for perfusion techniques. In addition, it is a dangerous anticoagulant to use when there are fresh wounds or partial defects of hæmostatic or coagulation factors. However, it is easily and quickly neutralised by either protamine sulphate or Polybrene and these can effectively stop heparin-induced hæmorrhage once it has started.

Weight for weight Polybrene was found to be more effective than protamine and, if either has toxic effects, then Polybrene is the drug of choice. Otherwise there does not appear to be any advantage in one over the other. The anticoagulant effect of both drugs appears to be similar, but the safety margin

is generous (4 mgm./100 ml. blood). Neither drug was found to produce toxic effects.

Fibrinolysis was very active in most cases, but was not found to be deleterious to the patient. Whether it was responsible for fibrinogen depletion, in the three cases described, is uncertain.

Such defibrination or defibrinogenation must be a potential danger, but, from studies of obstetrical patients (Sharp, Biggs, Howie and Methuen, 1958), such partial defects as have been encountered in these cases do not merit routine prophylactic treatment with concentrated fibrinogen as suggested by Nilsson and Swedberg (1958).

[illegible]

reappearing platelets are for the most part damaged, non-viable, and unable to survive. The platelet count only returns to normal when the patient produces a new generation of viable platelets.

Platelet transfusions are not necessary if no hæmorrhage takes place. But if it does, fresh blood containing viable platelets should be given at the end of perfusion in order to raise not only the level of platelets, but that of any other plasma factor that may be depleted. Fresh blood alone cannot control hæmorrhage when heparin remains unneutralised.

Hæmolysis was not a major problem in the present series. When increased, it appears to be solely an indicator of faulty perfusion technique, and was not a cause of any particular complication in the patient.

The use of profound hypothermia in the sequence of perfusion was not found to alter the blood picture apart from inducing more marked thrombocytopœnia. This procedure appears to reduce rather than increase the incidence of hæmorrhage.

The majority of the above statements are based on successful perfusions of moderate duration (1-1½ hours). Longer or inadequate perfusions seriously prejudice the patient's chance of survival and increase the incidence of severe hæmorrhage. In such cases, the blood picture does not differ in any significant way from that already described.

ACKNOWLEDGMENTS

We are indebted to Professor P. R. Allison and Mr A. J. Gunning, of the Nuffield Department of Surgery, without whose co-operation and interest this study would not have been possible, and to Miss Ann Kimbrey for technical assistance.

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of both these substances were performed by Dr. Blashko and Dr G. V. R. Born on blood samples obtained in [illegible]

significant amounts of these toxic substances were detected in the plasma.

Post-Operative Period.

The rise in platelet count that develops in the immediate post-perfusion period was not sustained [illegible]

cytopœnia was not accompanied by a prolonged bleeding time.

Heparin was not used post-operatively, but phenindione was given prophylactically in three cases. In all, the response to the initial dose was dramatic, the plasma "prothrombin" falling to levels below 8% (Owren and Aas, 1951). These low levels in association with the thrombocytopœnia already mentioned were accompanied by a prolongation of the bleeding time. In all cases it was felt that the risk of secondary hæmorrhage was too great to justify continuation of therapy. In two cases Vitamin K was given as an antidote, and in both the bleeding time returned to normal limits.

Discussion.

The greatest danger to the patient [illegible]

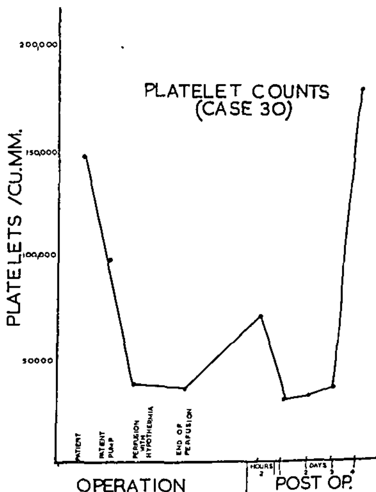


Figure 3—*Platelet counts* during perfusion in the post-operative period (case 30)

the outcome of successful surgery can be jeopardised by post-perfusion hæmorrhage from cut surfaces. This bleeding takes the form of a diffuse ooze from all cut surfaces rather than from only one bleeding point. The amount of blood lost can be a matter of pints within a few minutes It is surprising that such diffuse hæmorrhage is not the invariable result of perfusion as the composite defect induced by thrombocytopœnia, active fibrinolysis, partial defibrination, and probable partial depletion of other coagulation factors, such as prothrombin and antihæmophilic globulin would, in most hæmatologists' opinion, be an adequate explanation Yet these defects can exist together without hæmorrhage occurring

The factor responsible for this post-perfusion [illegible]

hage. Where heparin neutralisation [illegible] no hæmorrhage occurred apart from that from unsecured blood vessels.

The measurable loss of heparin from the blood during perfusion was initially interpreted to be due to destruction of the heparin (Seening, 1959), but if the antidote was given in amounts equivalent to [illegible] in the blood at [illegible]

[illegible] the normal circulation is restored. [illegible]

Heparin is an inefficient anticoagulant for preservation of stored blood, but it is the only one available for perfusion techniques. In addition, it is a dangerous anticoagulant to use when there are fresh wounds or partial defects of hæmostatic or coagulation factors However, it is easily and quickly neutralised by either protamine sulphate or Polybrene and these can effectively stop *heparin-induced* hæmorrhage once it has started.

Weight for weight Polybrene was found to be more effective than protamine and, if either has toxic effects, then Polybrene is the drug of choice. Otherwise there does not appear to be any advantage in one over the other. The anticoagulant effect of both *drugs appears to be similar, but the safety margin*

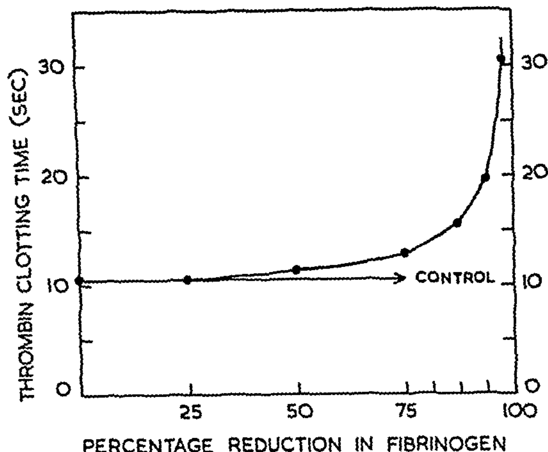


Figure 2—Effect of reduction in fibrinogen on the thrombin clotting-time. 0% reduction is equivalent to 300 mg. of fibrinogen per 100 ml of plasma.

unneutralised heparin

The heparin circulating after perfusion is derived from the heparinising dose given to the patient (2-2.5 mg./kg.) and the perfused donor blood (20-25 mg per 500 ml.), a variable amount of which is used during bypass. It is thus difficult after bypass to estimate accurately and quickly the amount of heparin requiring neutralisation. We have found it more rapid and convenient than assaying the heparin after perfusion, to give a dose of antidote based on the heparinising dose given to the patient, and thus related to body weight, and expressed as a ratio of antidote to heparin. If unneutralised heparin was detected after this, further doses of antidote were

experimental dogs after perfusion and to human volunteers without producing an inhibition of coagulation. There is a good margin of safety, as with the newer antidote Polybrene, in the doses

Abnormal Haemorrhage After Perfusion

THE IMPORTANCE OF UN-NEUTRALISED HEPARIN AND FIBRINOGEN DEPLETION

N. G. ROTHNIE and J. B. KINMONTH — St Thomas's Hospital Medical School, London

The last decade has seen an increasing number of congenital and acquired cardiac defects corrected by open operation using an extracorporeal circulation. One of the problems of recent cardiac surgery is a disturbance of natural hæmostasis leading to abnormal bleeding following total body perfusion. This is mainly due to the trauma inflicted on the blood as it passes through the external circuit and to the massive transfusion of donor blood pumped from the heart-lung machine. The heparin, essential *during perfusion*, may also cause bleeding if it is incompletely neutralised afterwards.

Abnormal bleeding after perfusion has been attributed to a deficiency of one or other of the clotting elements of the blood (Osborn et al 1955; Allen 1958; Brown and Smith 1958; Von Kaulla and Swan 1958; Gollub et al 1959; Perkins et al 1959 and Nilsson and Swedberg 1959). However, scant attention has previously been given to the role of unneutralised heparin and fibrinogen depletion in its causation. Under the guidance of Dr G. I. C. Ingram, we have studied the importance of these two factors, for which corrective treatment is readily available.

We used simple and rapid clotting tests which can be done by the heart-lung team at any time after perfusion:

1. The thrombin clotting time to detect unneutralised heparin.
2. An *absorptiometric method for estimation of* fibrinogen followed by observation of the fibrin clot for lysis.

Thrombin clotting-time (Ingram 1955; Rothnie and Kinmonth 1960).

This is a more sensitive indicator of heparin activity than the other clotting tests, e.g. the whole blood clotting time (Lee and White) and one-stage prothrombin time (Quick), used in previous studies.

Thrombin (bovine) and M/80 Ca Cl2 are added to citrated *platelet-poor* plasma and clot formation timed. The main factors affecting the thrombin clotting-time, apart from heparin, are the thrombin and fibrinogen concentrations. The test is made suitably sensitive to heparin activity by adjusting the thrombin concentration to give a control clotting-time of 10-12 sec. (Fig. 1).

It is important to know the effect of fibrinogen [illegible]

reduction is greater than that found after an average perfusion so that any increase in the thrombin clotting-time after operation is probably due to heparin. (Fig. 2).

This is confirmed by adding toluidine blue

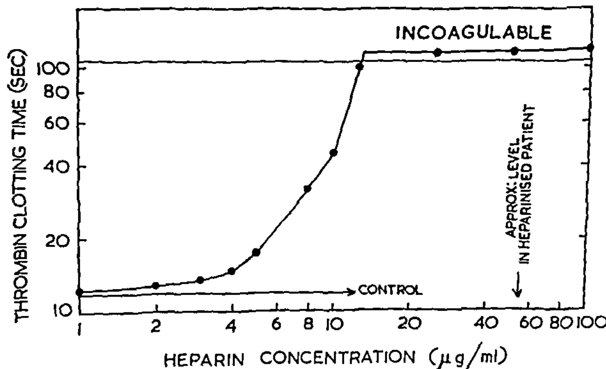


Figure 1—Effect of different heparin concentrations on the thrombin clotting-time

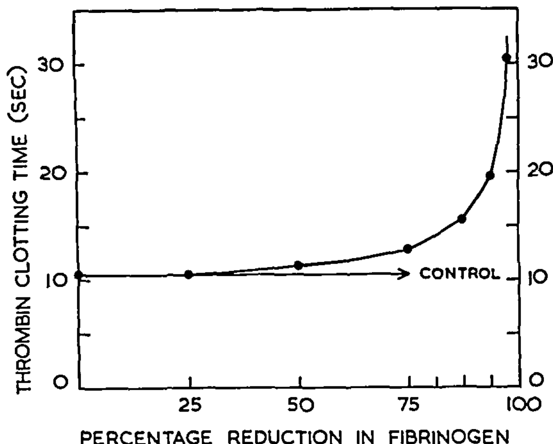


Figure 2—Effect of reduction in fibrinogen on the thrombin clotting-time. 0% reduction is equivalent to 300 mg of fibrinogen per 100 ml. of plasma

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unneutralised heparin and not to a marked decrease in fibrinogen

In our early cases, using protamine as the heparin neutraliser, troublesome oozing from the wounds

after perfusion, to give a dose of antidote based on th
heparinising dose given to the patient, and thu

a ratio of 1.5:1.

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Abnormal bleeding after perfusion has been ... er of the clot- ... et al 1955; ... ; Von Kaulla ... Perkins et al ... 9). However, scant attention has previously been given to the

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Thrombin (bovine) and M/80 Ca Cl2 are added to citrated platelet-poor plasma and clot formation timed. The main factors affecting the thrombin

100 mg.% or less, is necessary before there is prolongation in the thrombin clotting-time. This reduction is greater than that found after an average perfusion so that any increase in the thrombin clotting-time after operation is probably due to heparin. (Fig. 2).

This is confirmed by adding toluidine blue

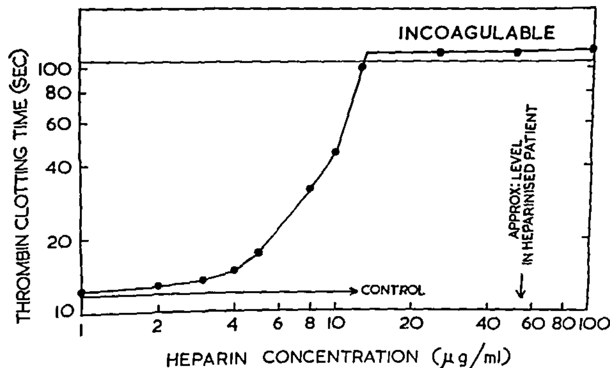


Figure 1—Effect of different heparin concentrations on the thrombin clotting-time.

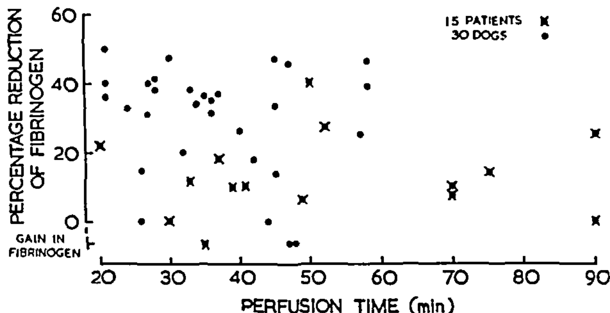


Figure 5—Scattergram relating to percentage reduction in fibrinogen to the duration of perfusion in fifteen clinical and thirty experimental perfusions.

E.B. AGE 13 Wt 33 Kg

CLOT LYSIS + + NIL NIL NIL

PLATELET COUNT (10^3/cu mm) 300 200 100 0

FIBRINOGEN CONCENTRATION (mg%) 300 200 100 0

TH.CL.T.(sec) 15 10 5 0

HEPARIN (2·5 mg/Kg)

PERFUSION

POLYBRENE (3·75 mg/Kg)

FIBRINOGEN INFUSION (4·4 Gm)

BEFORE PERFUSION 0 1 2 3 4 5 6 7

TIME (hours)

Figure 6—Changes in the platelet count (•——•) *fibrinogen concentration* (o——o) *and thrombin* clotting-time (TH.CL.T.) after perfusion for 2½ hours + indicates fibrinolysis The effect of an infusion of fibrinogen is shown

With the tests described, the detection and elimination of residual heparin was found to be an

The citrated platelet-poor plasma is recalcified and the opacity increase due to the fibrin clot ... ng a violet filter. ... is converted to fibrinogen con- ... absorptiometer ... calibrated with plasma containing known quantities of fibrinogen

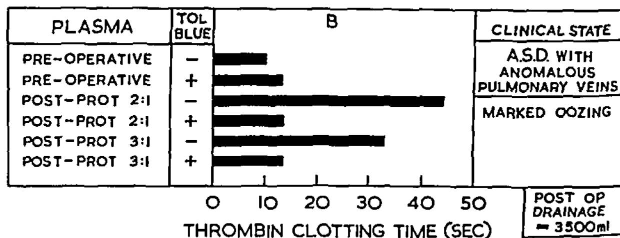


Figure 3—A patient with atrial septal defect (A S D.) and anomalous pulmonary veins showing the use of the thrombin clotting-time in the detection of unneutralised heparin, which caused abnormal bleeding after perfusion

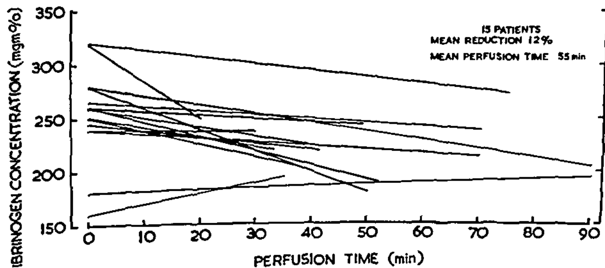


Figure 4—The changes in fibrinogen concentration in fifteen clinical perfusions plotted to show the rate of change in individual cases

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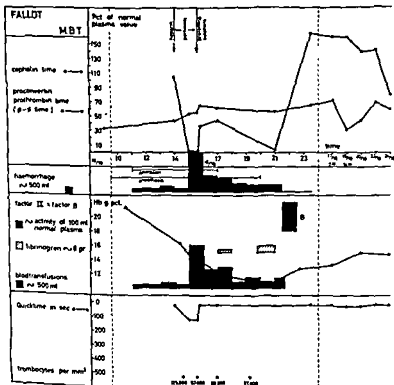
Coagulability During Extracorporeal Circulation With Particular Reference To Antihaemophilic Factor B (Factor IX)

J. MATZKE, R. SMITH JENSEN and I. H. RYGG—The University Hospital and the State Serum Institute, Copenhagen

We have tried to find the reasons for the serious bleeding tendency often encountered during and after extracorporeal circulation It is usually not explained by the platelet count, bleeding time, P and P test, whole blood clotting time, fibrinogen assay and tests for fibrinolysis.

Our observations refer to four patients with Fallot's tetralogy and two with atrial septal defect. The heart-lung machine used was the Rygg-Kyvsgaard type, in which a plastic bag is used as oxygenator and discarded after use.

The patients' clotting factors were studied by the following methods: (1) The cephalin time of Waaler (1957), which is sensitive to deficiency of [illegible] (Owren and Aas, 1951) and (3) the Quick test. (4) In some cases the proaccelerin (factor V) test (Hjort, 1957) was carried out.



The cephalin time was used as a screening test for the factors mentioned, after an evaluation of the other three tests When a prolonged cephalin time was found, the following additions were made in turn to the reagents: barium sulphate-adsorbed plasma, containing factor VIII and PTA; old serum, containing factor IX and PTA; adsorbed serum, containing only PTA For measurement of factors VIII and IX, modifications of the methods of Waaler (1959) and Stapp (1958) were used. When a deficiency of factor IX was found, the modifications of the cephalin test mentioned were used to measure the factor IX level.

In the two ASD patients, the initial tests were all normal. During heparinisation the cephalin tests fell to under 1%, from a marked decline in factor IX, and the Quick time was prolonged owing to the antithrombin action; the P and P test was less sensitive to this action, being normal. Factor V was depressed, but not enough to cause this prolongation in the Quick test. After protamine sulphate the tests were all normal, but in one-and-a-half hours a secondary fall was found, particularly a great decline in the cephalin test and factor IX. There is at present no adequate

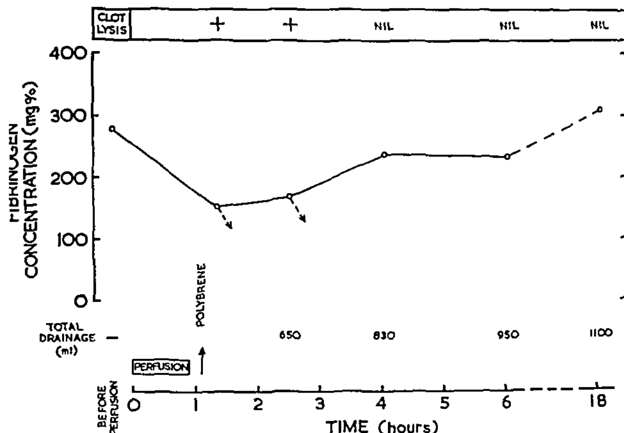


Figure 7—Changes in fibrinogen concentration (o——o) after perfusion for 50 min + indicates fibrinolysis Post-operative blood drainage from the chest is shown.

no direct relationship between the degree of reduction of fibrinogen and the duration of perfusion (Fig. 5). The degree of reduction depends on many variable factors which differ from case to case. One cannot therefore forecast accurately the changes due to perfusion. Changes in fibrinogen were insufficient to produce a failure of hæmostasis in these patients and none showed evidence of abnormal oozing or fibrinolysis.

fibrinogen concentration—ca. 100 mg.% (Fig 6). The plasma clot showed rapid lysis (within 20 min.). A plasma specimen half an hour later produced scanty clot which was rapidly lysed. There was marked oozing from the various incisions An infusion of 4.4 g. of human fibrinogen in 200 ml. of distilled water was given with a return to normal fibrinogen concentration as estimated at intervals over the following 3 hours. There was no evidence of fibrinolytic activity after the infusion and the thrombin

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showed

briefer and less marked fibrinolytic activity and fibrinogen depletion, and post-operative blood drainage was not excessive.

circulating fibrinogen.

There is at present no satisfactory method of reducing fibrinolytic activity and treatment consists of fibrinogen replacement until activity ceases and a normal fibrinogen concentration is attained.

hours before the operation and heparinised (75 mg. heparin for 400 ml. blood). All patients received

results of a protamine or Polybrene titration test. A series of 10 tubes, each containing 0.1 ml. of the

Figure 1

Clotting time of 10 patients before the second dose of Heparin - antidote

Figure 2

explanation of this. Two hours after perfusion there was a compensatory phase of hypercoagulability.

In the four patients with Fallot's tetralogy serious bleeding occurred before, during and after perfusion and was not controlled by heparin-neutralisers, blood transfusion or surgical hæmostasis. The patients were treated with a human plasma preparation made from Cohn's fraction IV. This contains several blood clotting factors, in particular factor IX.

The first and second of these patients, though the initial P and P and cephalin times were above the bleeding limit, bled heavily before perfusion and after heparin a very severe hæmorrhage occurred (Fig.). The cephalin test fell to under 1%, and the Quick time was prolonged to over 100 seconds. After protamine sulphate the cephalin time was less and the Quick time normal The secondary rise in the cephalin time was found here also, with a corresponding severe and prolonged bleeding since the spontaneous hypercoagulable phase did not appear. After infusion of the factor IX preparation, the cephalin time was shortened, with corresponding hæmostasis. The Quick time showed no secondary prolongation, presumably because there was no fibrinogen deficiency nor prolonged heparin effect. As expected, infusion of fibrinogen had no effect on the bleeding.

The third patient also had an initially prolonged [illegible]

[illegible] for a short time after the heart was entered, it had stopped [illegible]

[illegible] effective before but not after perfusion. The factor IX activity is highest at a pH of 7.35, being nearly inactive at 7.0.

In the fourth patient the findings were similar to the first, but we tried to avoid the secondary prolonged cephalin time by infusing factor IX along with the second injection of protamine sulphate. This infusion was repeated, with a decrease in the cephalin time and cessation of the heavy blood loss

The common finding in all these patients was a secondary [illegible]

[illegible] great value.

Heparin seems particularly to influence the factor IX level, and to ensure an effective anticoagulant effect during perfusion it is advisable to prolong the Quick time in order to utilise the antithrombin action. The P and P test is virtually unchanged throughout, and is not useful in this context.

In uncomplicated cases, where the hypercoagulable phase comes soon, it may be better to start anticoagulant treatment earlier in the post-operative period.

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Blood Coagulation Problems During Extracorporeal Circulation In Man

M VERSTRAETE, A AMERY and C. VERMYLEN — Department of Medicine, University of Louvain, Belgium

Bleeding has been a major problem in the use of extracorporeal circulation in dogs and in man The hæmorrhagic state, sometimes found in man [illegible]

[illegible] from one group to the other, it is generally accepted that the better results are a consequence of a careful routine and greater experience on the part of the [illegible]

[illegible] bleeding is a troublesome [illegible]

[illegible] Brown Harrison blood heat exchanges assembly was interposed in the inflow [illegible]

DISCUSSION

Dr BIGGS — Dr Matzke is to be congratulated on his discovery of a test, the cephalin time, which appears to give results which are correlated with the tendency of the patient to hæmorrhage. It is a pity that the results of this test have been interpreted as showing factor IX deficiency. The measurement [illegible] indeed, by the coagulability of fibrinogen Even correction of the clotting time by aged serum will not ensure that the technique gives a measure of factor IX. Thus, while Dr Matzke's results are very helpful in revealing a test which may correlate with the bleeding tendency it may prove misleading if the results are interpreted too narrowly at this stage as indicating a specific defect in factor IX.

Dr SAMAMA — I should like to describe a study of a patient who died from cataclysmic fibrinolysis during operation for tetralogy of Fallot. It throws light on the failure of substitute therapy in superacute fibrinolysis, and verifies the efficacy of a new antifibrinolytic agent C 766 or Iniprol.

The patient was a girl aged 17. Before operation there was only a slight thrombocytopœnia and no [illegible] after 6 hours

Laboratory tests showed only traces of fibrinogen in the plasma. Quick's test in the presence of fibrinogen was 50% (factor V 40%). Von Kaulla's euglobulin technique showed that lysis was almost immediate, while a mixture of patient's plasma with 3 parts of normal plasma gave a lysis time of 15 minutes (normal over 180 minutes). Thrombo-elastography was characteristic of superacute fibrinolysis and showed that the patient's plasma was capable of lysing 10 times its own volume, i e the patient's total plasma volume was capable of lysing fibrinogen in the order of 100 gms One can readily understand the failure of the standard substitute treatment.

In vitro, with 100 units of inhibitor, the lysis time increased from 45 to 150 minutes; with 200 units, lysis was practically abolished. It seems that a clinical dose of 5 million units would attenuate or abolish the residual lytic activity

Professor OWREN — As stated by Sharp, the oozing cannot usually be accounted for by the moderate [illegible] deficiency [illegible] The mech[illegible] draw your [illegible] may play a role at least in certain cases, namely a [illegible] This [illegible] time [illegible] vivo [illegible] cand[illegible] -come [illegible] regarding the required dosage.

The thrombocytopœnia is largely caused by trapping of platelet clumps in the filter. I believe that the main reason for platelet clumping is the release of factor R by contact of red cells with foreign surfaces. Even very small amounts of factor R drastically increase platelet adhesiveness and clumping. Intravenous injection of factor R in rabbits causes, as mentioned previously, platelet clumping and thrombocytopœnia, presumably because of trapping of platelet clumps in the capillary bed. However, factor R becomes inactivated by plasma, the clumping is reversed, and the platelets reappear in the circulation. This observation in rabbits is much like the temporary thrombocytopœnia of by-pass circulation as shown by Sharp, and the mechanisms may be the same. The thrombocytopœnia of by-pass circulation may be largely prevented by devising pumps and using plastic tubes which give minimal trauma to the red cells. Further, the filter could probably be discarded if its only effect is to remove small platelet clumps, because the introduction of small aggregates of platelets into the circulation seems to be without harmful effect. The clumps are broken down and the platelets are released after a temporary trapping.

Thromboplastin generation test							Thrombin clotting time
Addition	1	2	3	4	5	6	13
Saline	11	11	11	11	11	11	
1/32 Polybrene	180+	180+	180+	180+	180+	180+	14
1/128 Polybrene	155	155	158	151	150	147	14
1/256 Polybrene	50	20	17	15	15	15	14
1/512 Polybrene	14	13	12	12	12	12	13

Clotting time in seconds

Figure I

injected. A second series of the anti-heparin agent with lower concentrations (from 2 gamma/ml. to 20 gamma/ml. with 2 gamma intervals) was used after the first neutralisation for further detection of traces of heparin.

Other techniques used were the Lee and White clotting time of venous blood, fibrinogen assay by the method of Clauss (1957) and fibrinolysis observed

results obtained in cases of aortic stenosis explain the growing frequency of open heart surgery with cardiac by-pass in adults. Half of the patients had a ventricular septal defect. It is to be noted that patients with pulmonary stenosis and atrial septal defects

The plasma fibrinogen was also decreased at the end of the cardiac by-pass (to about 140 mg. %) and a tendency to a further slight fall was noted in some patients. If the results of all cases are averaged, there is a 10 mg. per cent difference observed immediately after the slow administration of protamine

was needed in the post-operative period (15 cases). There were 10 cases where two injections of protamine sulphate were given (Fig. 1). In 3 cases a supplementary dose of protamine sulphate was given mainly because the surgeon felt that the patient was bleeding excessively, although the clotting time and the protamine titration test were normal. If the amounts of protamine given are averaged, it was found that the protamine-heparin ratio was only 1.1 in these 10 cases. The fact that a second dose of heparin antidote was needed in this group suggests that the optimal protamine-heparin ratio

3 mg. heparin/3 mg. Polybrene, an immediate and sustained normalisation of the clotting time was obtained. The results were as favourable with

10, 25 and 50 gamma heparin/ml. (Fig. 2). It can be seen that in similar experiments with protamine sulphate, a rather limited range of concentration corresponds to normal coagulation times; higher or lower concentrations give prolonged clotting times because all the heparin is not neutralised or too much protamine is added and exerts an anti-

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DISCUSSION

Dr BIGGS — Dr Matzke is to be congratulated on his discovery of a test, the cephalin time, which appears to give results which are correlated with

Dr SAMAMA — I should like to describe a study of a patient who died from cataclysmic fibrinolysis during operation for tetralogy of Fallot. It throws light on the failure of substitute therapy in superacute fibrinolysis, and verifies the efficacy of a new antifibrinolytic agent C 766 or Iniprol.

The patient was a girl aged 17. Before operation there was only a slight

after 6 hours.

Laboratory tests showed only traces of fibrinogen in the plasma. Quick's test in the presence of fibrinogen was 50% (factor V 40%). Von Kaulla's euglobulin technique showed that lysis was almost immediate, while a mixture of patient's plasma with 3 parts of normal plasma gave a lysis time of 15 minutes (normal over 180 minutes). Thrombo-elastography was characteristic of superacute fibrinolysis and showed that the patient's plasma was capable of lysing 10 times its own volume, i.e. the patient's total plasma volume was capable of lysing fibrinogen in the order of 100 gms. One can readily understand the failure of the standard substitute treatment.

In vitro, with 100 units of inhibitor, the lysis time increased from 45 to 150 minutes; with 200 units, lysis was practically abolished. It seems that a clinical dose of 5 million units would attenuate or abolish the residual lytic activity.

Professor OWREN — As stated by Sharp, the oozing cannot usually be accounted for by the moderate

is disclosed by prolongation of the bleeding time and reduced platelet adhesiveness by the in vivo technique (Borchgrevink, Chr.: Acta Med. Scand. 1960, in print). Platelets in donor blood become

regarding the required dosage.

The thrombocytopoenia is largely caused by trapping of platelet clumps in the filter. I believe that the main reason for platelet clumping is the release of factor R by contact of red cells with

However, factor R becomes inactivated by plasma,

released after a temporary trapping.

Thromboplastin generation test							Thrombin clotting time
Addition	1	2	3	4	5	6	13
Saline	11	11	11	11	11	11	
1/32 Polybrene	180+	180+	180+	180+	180+	180+	14
1/128 Polybrene	155	155	158	151	150	147	14
1/256 Polybrene	50	20	17	15	15	15	14
1/512 Polybrene	14	13	12	12	12	12	13

Clotting time in seconds

Figure 1

injected. A second series of the anti-heparin agent with lower concentrations (from 2 gamma/ml. to 20 gamma/ml. with 2 gamma intervals) was used after the first neutralisation for further detection of traces of heparin.

The majority of patients were infants; the good results obtained in cases of aortic stenosis explain the growing frequency of open heart surgery with cardiac by-pass in adults. Half of the patients had a ventricular septal defect. It is to be noted that patients with pulmonary stenosis and atrial septal defects

injection of protamine sulphate. When the results of all patients were grouped, however, no difference could be noted.

ately after the slow administration of protamine

was based on the protamine or Polybrene titration test. On retrospective analysis of the quantities used, it was found that the protamine sulphate-heparin ratio was 1.5 and the Polybrene-heparin ratio 1.4 for all cases where only one dose of heparin antidote was needed in the post-operative period (15 cases) There were 10 cases where two injections of protamine sulphate were given (Fig. 1). In 3 cases a

the amounts of protamine given are averaged, it was found that the protamine-heparin ratio was only 1.1 in these 10 cases. The fact that a second dose of heparin antidote was needed in this group suggests that the optimal protamine-heparin ratio

sustained normalisation of the clotting time was obtained. The results were as favourable with 5 mg. heparin/5 mg. Polybrene per kg. body weight

With a 2/1 Polybrene-heparin ratio, no fall

advantage of Polybrene over protamine sulphate is the finding that in vitro a wide range of concentrations neutralises a fixed amount of heparin for 10, 25 and 50 gamma heparin/ml. (Fig 2). It can be seen that in similar experiments with protamine sulphate, a rather limited range of concentration corresponds to normal coagulation times; higher or lower concentrations give prolonged clotting times because all the heparin is not neutralised or too much protamine is added and exerts an anticoagulant activity by itself. A final advantage is that with Polybrene no fall in blood pressure or agglutination of red cells is observed in the doses used even after rapid intravenous administration.

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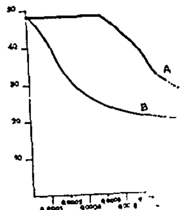
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knowing whether these assays represent the truth, in view of the possible artefact in respect to the antithrombin VI phenomenon of Kowalski. The only dissimilarity is the fall in the platelet count following bypass whereas there is no fall following the streptokinase infusion. This can be related to the mechanical loss of platelets during the bypass.

The development of a laboratory defect is a constant finding after bypass. Occasionally there is no abnormal bleeding and very occasionally it is catastrophic. More often there is abnormal bleeding as manifest by the blood coming through the chest drains, which goes on for several hours but is not of sufficient magnitude to cause anxiety.

NEUTRALIZATION OF HEPA- BY PROTAMIN-



... has passed. ... the use of ... inhibitor of plasminogen activation.

Dr SHARP — I should like to ask a question about the amount of heparin used in the perfusion blood. I feel that the platelet clumping and resultant thrombocytopœnia seen during perfusion are due to the heparin levels used being too low to prevent ...

... levels of heparin during perfusion.

... may explain the lack of serotonin release mentioned by Sharp.

... after streptokinase and plasmin therapy. At the same time, to prevent a new thrombotic tendency, heparin has to be given immediately.

I should like to ask Dr Matzke what concentration of factor IX he obtained in the concentrate given to the patients he described. He got quite a large increase in factor IX activity after administration of the concentrate ... of factor I... we had gr... factor IX ... normal pla... be due to ...

We k... Hagen... possib... Dr M... factor ... an incre... centrate ... the reas...

Dr NI...

Dr SHARP — I agree with Professor Owren that factor R does exist in hæmolysates and does clump platelets, but we have not noticed increased clumping or thrombocytopœnia in those cases where hæmolysis was excessive.

With regard to Dr Douglas's remarks, it has been our experience that fibrinolysis disappears within 2 hours of perfusion and in the one case where defibrination existed but was not treated the ... in about 6 hours ... has passed the ... ing secondary ...

I wonder whether the concept of neutralising ... fibrinolytic drugs like epsilon- ... fibrinolysis may ... neutralisation may ... mbi. If lysis is

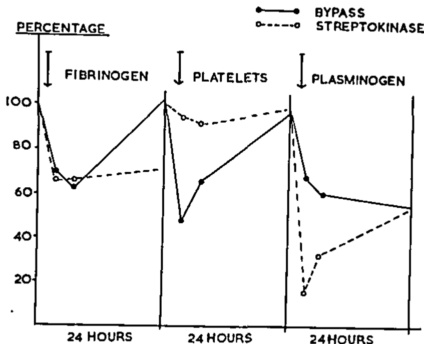


Figure 2

Dr DOUGLAS — The hæmostatic defect which

As has been stressed today it is certainly correct to study initially the question of proper neutralisation of heparin by protamine or Polybrene. I am not certain that I know how to ensure that the heparin / protamine titration has been accurately accomplished. The thrombin clotting time and the whole blood coagulation time techniques have serious limitations. It can be shown that Polybrene, protamine and heparin are all inhibitory to thromboplastin generation at a concentration which does not interfere with the thrombin clotting time of the plasma (Fig 1) The whole blood clotting time has the same type of technical limitation. I think that we have to use the thrombin clotting time and the whole blood clotting time in our attempts to judge the efficacy of the neutralisation effect, but it is probably wise to be aware of their limitations

In an attempt to sort out the nature of the hæmostatic abnormality there are a number of collateral methods of approach Dogs can be put on the bypass without heparin or protamine and they develop a hæmorrhagic state. The dog, of course, has a considerable capacity under specific circumstances for endogenous production of heparin, but this nevertheless is a pointer that this defect is more complex than merely a question of heparin neutralisation. Another pointer, which I think can help to disentangle the heparin/protamine issue from the remainder of the problem, is the fact that there is often a defect many hours after the operation when, even if no heparin antagonist had been given, one would have expected the heparin in any case to have disappeared in virtue of its usual "in vivo" survival time. The fibrinogen fall may on occasion be progressive for nine hours after the bypass. More often the initial fall is sustained despite the continued transfusion of fresh blood. This supports the concept that there is a continuing abnormality, which is probably proteolytic in its nature. Most of the reports today have shown only the occasional occurrence of increased fibrinolytic activity. These determinations have, however, been made on the basis of whole-clot lysis or lysis in dilutions of plasma Before the possible importance of proteolytic activity is decided it should be studied by more modern and sensitive techniques—the use of tagged fibrinogen and plasminogen-enriched tagged fibrinogen. Fig. 2 shows the mean results from 12 bypass operations and 4 streptokinase infusions It will be seen that with the bypass procedure there is an appreciable fall in plasminogen concentration

Some collateral evidence for the proteolytic nature of the bypass defect can be found in a comparison with the defect following streptokinase infusion (Figs. 2 and 3). I have some difficulty in

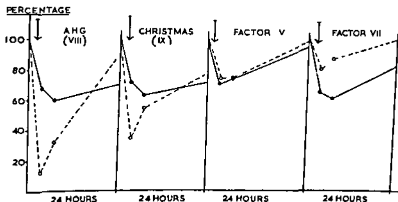


Figure 3

knowing whether these assays represent the truth, in view of the possible artefact in respect to the antithrombin VI phenomenon of Kowalski. The only dissimilarity is the fall in the platelet count following bypass whereas there is no fall following the streptokinase infusion. This can be related to the mechanical loss of platelets during the bypass.

The development of a laboratory defect is a constant finding after bypass. Occasionally there is no abnormal bleeding and very occasionally it is catastrophic. More often there is abnormal bleeding as manifest by the blood coming through the chest drains, which goes on for several hours but is not of sufficient magnitude to cause anxiety.

In the therapy of this condition it is important in the first place to do what one can to ensure adequate heparin neutralisation, and to be prepared to transfuse with fresh blood until the "proteolytic storm" has passed. There is a possibility for the future in the use of epsilon-amino-caproic acid as an inhibitor of plasminogen activation.

Dr SHARP — I should like to ask a question about the amount of heparin used in the perfusion blood. I feel that the platelet clumping and resultant thrombocytopœnia seen during perfusion are due to the heparin levels used being too low to prevent

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may explain the lack of serotonin release mentioned by Sharp.

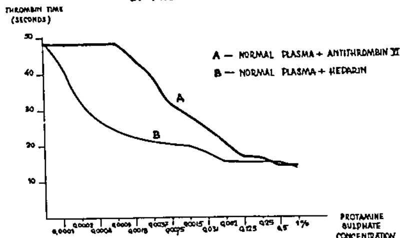


Figure 4

We know from Dr Niewiarowski's paper that Hageman factor is depressed by fibrinolysis. It is possible that the factor IX depression found by Dr Matzke was due to a depression of Hageman

Dr NIEWIAROWSKI — The Hageman factor

protamine titration. In an experiment, heparin and antithrombin VI were added to human plasma to

necessary than for neutralisation of heparin (Fig. 4).

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With regard to Dr Douglas's remarks, it has been our experience that fibrinolysis disappears within 2 hours of perfusion and in the one case

I wonder whether the concept of neutralising fibrinolysis with antifibrinolytic drugs like epsilon-amino-caproic acid is well-advised. Fibrinolysis may be more often beneficial, and neutralisation may induce unwanted intravascular thrombi. If lysis is

obviously doing harm, such treatment must, of course, be given.

Mr ROTHNIE — I would like to stress that abnormal bleeding may not only be due to hæmostatic defects but to surgical causes. Meticulous surgical hæmostasis is as essential after perfusion as carrying out clotting tests.

I am most interested to learn from Professor Owren about factor R and platelet clumping, and *relieved to hear that such clumps are reversible.* I assure him that we do not use the filter in the bypass circuit to remove the platelet clumps but to stop foreign material entering the patient's arterial circulation. Fortunately platelets are the only material we have found on the filter; one day we may have the courage to dispense with it, as he suggests, and so reduce the platelet loss. However, such platelet loss as we have found does not seem to have any serious effect on hæmostasis.

We have not found, as Dr Douglas has, that fibrinolysis lasts longer than a few hours after the end of perfusion. It seems to burn itself out gradually in 2-3 hours, and our object in treatment has been [illegible] infusion [illegible] ases. We [illegible] suggested [illegible]

Dr MATZKE — So far we have no adequate explanation for the phenomena I described My report is a preliminary one, but more extended investigations now in progress seem to show the same results. These are, I know, surprising, but I [illegible]

[illegible]

[illegible] a great [illegible] eated with [illegible] myeloma [illegible] of course, these patients did not have a major operation.

We seem to use larger amounts of heparin (3 mg. per kg) than the other investigators. I do not know whether this has a bearing on our better results in cardiac surgery, in terms of avoidance of platelet-clumping or intravascular coagulation.

In dogs undergoing a cardiac bypass antithrombin VI may appear but also another antithrombin not adsorbed by barium sulphate.